The American Journal of Medicine

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Change of address must reach us one month preceding month of issue.

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relief in minutes . . . Tedral brings symptomatic relief in a matter of minutes. Breathing becomes easier as Tedral relaxes smooth muscle, reduces tissue edema, provides mild sedation.

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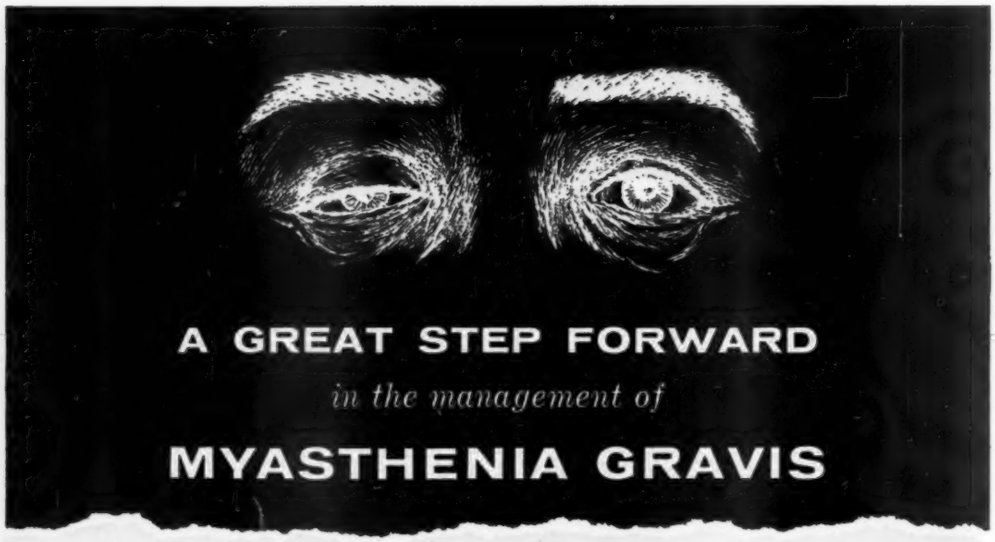

A GREAT STEP FORWARD

in the management of

MYASTHENIA GRAVIS

Mytelase chloride is a new antimyasthenic compound which, chemically and in clinical action, is quite different from older cholinergics.

Mytelase chloride in laboratory tests is "...five to ten times more active than neostigmine in anticholinesterase effect and has a duration of effect which is much more prolonged than that of neostigmine or Mestinon."[1] Patients "...feel better, are stronger, and are closer to their normal health."[2]

Some patients can replace their former 10 to 60 tablets daily with as few as 2 tablets of Mytelase.[1] As a rule Mytelase ". . . has roughly twice the effectiveness per milligram of neostigmine in reducing the symptoms of myasthenia gravis, and approximately twice the duration."[3] With the new drug sleep need not be interrupted for dosage.[2]

While Mytelase chloride exerts a more powerful and prolonged antimyasthenic effect, it has less cholinergic action on the gastrointestinal tract.[3,4] Patients thus find it more pleasant as maintenance therapy. With Mytelase "no chronic toxic effects to the kidneys, liver, or blood-forming organs were found . . ."[2]

Supplied: 10 mg. and 25 mg. tablets, bottles of 100.

Write for leaflet giving detailed clinical experience, discussion of dosage, side effects and cautions to be observed.

1. Westerberg, Martha R.; and Magee, K.R.: Myasthenia Gravis. *Neurology*, 5:728, Oct., 1955. 2. Schwab, R.S.; Marshall, Clare K.; and Timberlake, William: Win 8077 in Treatment of Myasthenia Gravis. *J.A.M.A.*, 158:625, June 25, 1955. 3. Schwab, R.S.: Win 8077 in the Treatment of 60 Myasthenia Gravis Patients. *Am. Jour. Med.*, 19:734, Nov., 1955. 4. Schwab, R.S.: What Is New in Myasthenia Gravis. *Current Med. Digest*, 22:35, July, 1955.

NEW YORK 18, N. Y.

Mytelase, trademark

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The American Journal of Medicine

Vol. XXI SEPTEMBER, 1956 No. 3

Editorial

Clinical Studies

The action of insulin is closely linked with its metabolic fate in the body and it is, therefore, a matter of some importance to trace its disposition from the time of injection. This can be done conveniently by labeling insulin with I-131, on the assumption that no essential alteration is thereby effected. The present study establishes that the plasma disappearance time of such labeled insulin is significantly prolonged in those subjects who have previously received insulin therapy, whether diabetic or non-diabetic, because of unduly firm binding to a plasma protein, perhaps in the nature of an antigen-antibody reaction. The biologic activity and metabolic degradation of insulin consequently are affected. This is one of the many factors determining the insulin requirement and presumably plays a role in the development of insulin resistance.

It has never been made clear why and how patients in diabetic coma tolerate doses of insulin more than sufficient to cause fatal hypoglycemia in the same subject when not in acidosis; a temporary form of insulin resistance which usually disappears within a few days after relief of the acidotic episode. This problem is attacked in the present study by the *in vitro* rat hemidiaphragm technic. Some of the prevailing hypotheses, that the humoral insulin inhibitor in question is related to acidosis *per se*, to increase in plasma adrenocortical steroids, to specific antibodies and the like found no support in the results obtained. No alternative mechanism, however, is demonstrated.

It has long been accepted that a division of diabetic subjects to express apparent differences in insulin sensitivity is warranted by clinical expediency and other considerations but the criteria for differentiation between "stable" and "brittle" diabetic subjects are arbitrary and often unsatisfactory. The present authors propose new ways to establish old criteria and make some interesting

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NEW CONCEPT IN URINE-SUGAR TESTING

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specific enzyme test for urine glucose

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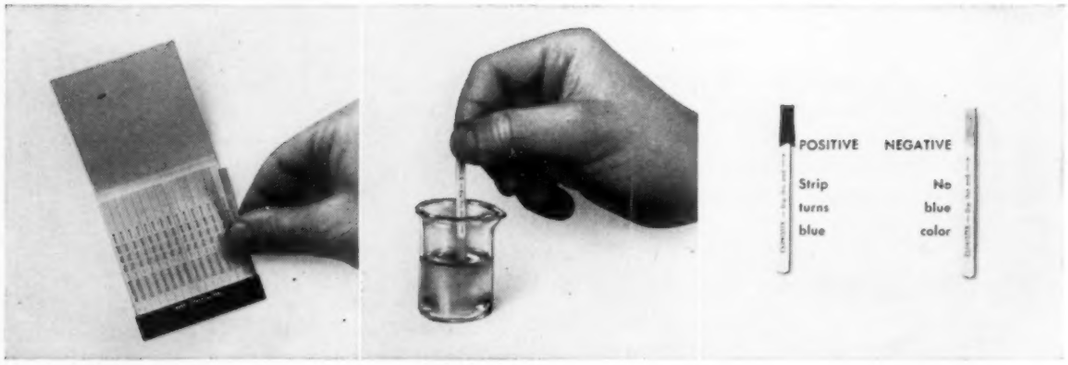

complete specificity...unaffected by non-glucose reducing substances...differentiates glucose from other urine-sugars... thousands of tests reveal no substance causing a false positive.

extreme sensitivity...detects glucose concentrations of 0.1% or less.

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VOLUME TWENTY-ONE NUMBER THREE

points in the process, some relating to the long controversy over completeness of control. Many issues of this discussion are open to debate but few, probably, would argue against the implication that what we call diabetes mellitus is a composite of two or more distinct metabolic disorders and that these differences are reflected in brittle and stable diabetic subjects.

The authors had the unusual opportunity to study the clinical consequences and the replacement requirements of surgically induced adrenal cortical deficiency in forty-four patients subjected to total or subtotal adrenalectomy for intractable hypertension. The status of such patients may differ in important respects from that of Addison's disease, and these differences throw light on adrenal cortical function both in the normal subject and in patients with Addison's disease. The totally adrenalectomized patient apparently can be maintained indefinitely by a regimen of cortisone or DCA or both, but is apt to manifest simultaneous indications of both hypo- and hyperfunction of the adrenal cortex.

Using such stimuli as salt deprivation, exhibition of ACTH and inhibition of endogenous ACTH secretion by cortisone administration, the authors demonstrate distinct mechanisms for regulation of the excretion of aldosterone and of hydrocortisone; aldosterone excretion being determined predominantly by the water and electrolyte equilibrium of the body, hydrocortisone excretion by ACTH. These findings in normal man are supported by clinical studies in hypopituitarism, congestive failure, nephrosis and cirrhosis. The mechanisms by which electrolyte and water balance control aldosterone retention independently of ACTH secretion remain to be elucidated.

Review

Filter paper electrophoresis has won rapid and wide acceptance as a routine and research tool for analysis of the serum proteins. Dr. Durrum and his colleagues, who pioneered in the technical and analytic phases of development of this new method, now come forward with the first broad survey of the results obtained over the years in a large general hospital. These results, both as to spectra of distribution of the serum proteins in various diseases and the clinical significance of abnormalities in particular fractions, conform in general with the findings and conclusions based on prior separations by salt and ethanol fractionation or moving boundary electrophoresis. The study is not intended to shed additional light on the serum proteins in diseases of special interest in this connection, but rather to indicate the scope and limitations of the procedure as a general method for the separation of the serum proteins and this it does admirably and authoritatively.

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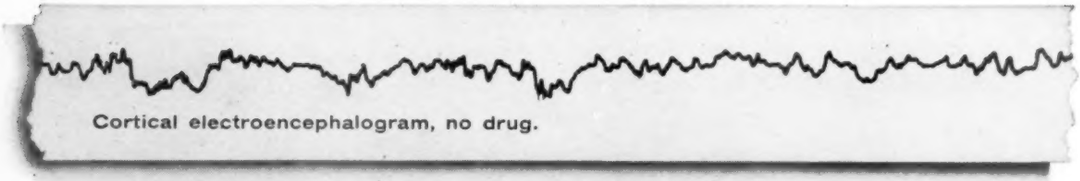
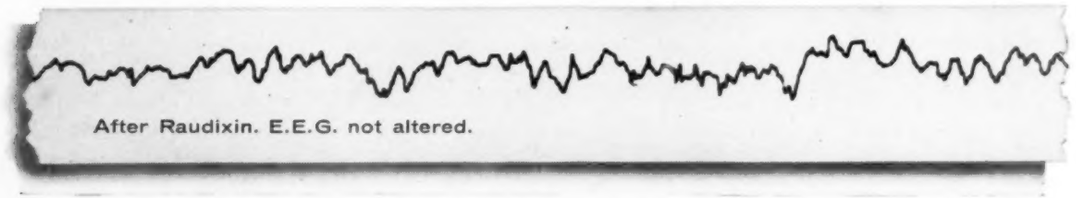
comparison of the effect of RAUDIXIN (tranquilizer) and a barbiturate (sedative)

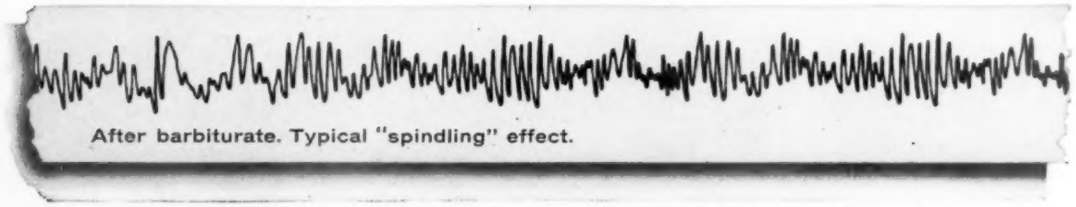


Cortical electroencephalogram, no drug.



After Raudixin. E.E.G. not altered.

Raudixin acts in the area of the midbrain and diencephalon and does not depress the cerebral cortex, as can be seen in this electroencephalogram. Consequently, the tranquilizing effect of Raudixin is generally free of loss of alertness.



After barbiturate. Typical "spindling" effect.

Because barbiturates and other sedatives depress the cerebral cortex, as indicated by this "spindling," the sedation is often accompanied by a reduction in mental alertness.

RAUDIXIN

SQUIBB WHOLE ROOT RAUWOLFIA SERPENTINA

SQUIBB

DOSAGE: Usual initial dosage is 200 mg. daily. Maintenance dosage may be adjusted within a range of 50 mg. to 500 mg. daily, depending on the response observed and the possible appearance of side effects. Most patients can be adequately maintained on 100 mg. to 200 mg. per day. Because of its sustained action, Raudixin may be given in single daily doses if desired. *Note:* Tranquilizing action is usually evident in 3 to 10 days; for a more rapid onset of effect, the patient may be given a priming dose of 200 to 300 mg. twice daily for the first 3 days.

SUPPLY: 50 mg. and 100 mg. tablets, bottles of 100, 1000 and 5000.

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Seminar on Diseases of the Pancreas

The systemic disorder which has come to be known as cystic fibrosis of the pancreas involves more than disease of the pancreas but is an important cause of pancreatic deficiency in children. The basic anomaly seems to be a genetically determined defect of mucous secretions in many organs of the body. This defect is most conspicuous in the pancreas, causing cystic fibrosis of that organ; the lungs, causing bronchial obstruction with secondary infection; the gastrointestinal tract, causing meconium ileus; the liver, causing cirrhosis with portal hypertension; and the sweat glands, causing excessive loss of sodium chloride and salt depletion. Not all these organs are uniformly involved, of course, and larval forms of the disease appear to be not uncommon. We have here again an example of how insight into pathogenesis of a disease makes possible more intelligent understanding and more effective treatment of the underlying disorder.

Combined Staff Clinic

Combined Staff Clinics (Columbia University College of Physicians and Surgeons)—This Conference begins by recounting the tortuous development of concepts of edema formation, indicating how, as each factor in pathogenesis was discovered, it was assigned an exclusive role, in the familiar pattern of the story of the blind men of India and the elephant. The Starling principles, backward and forward failure, the role of the kidney in retention of sodium and water, the part played by glomerular filtration and tubular reabsorption, the action of aldosterone and the role of secondary aldosteronism—all are described in historic and conceptual perspective. Dr. Gilman then contributes a characteristically lucid discussion of the problems of mobilization of edema fluid and the modes of action of diuretic agents. Out of the conference as a whole comes clearer insight into the mechanisms and management of edema which should enable the physician to give to his patient that individualization of treatment which, as Dr. Gilman declares, is required for the most skillful therapy.

Clinico-pathologic Conference

Clinico-pathologic Conference (Washington University School of Medicine).

Case Reports

A well studied case of unusual interest in which "juvenile" cirrhosis was associated with symptoms and signs suggesting the presence of pulmonary arteriovenous fistulae which, however, could not

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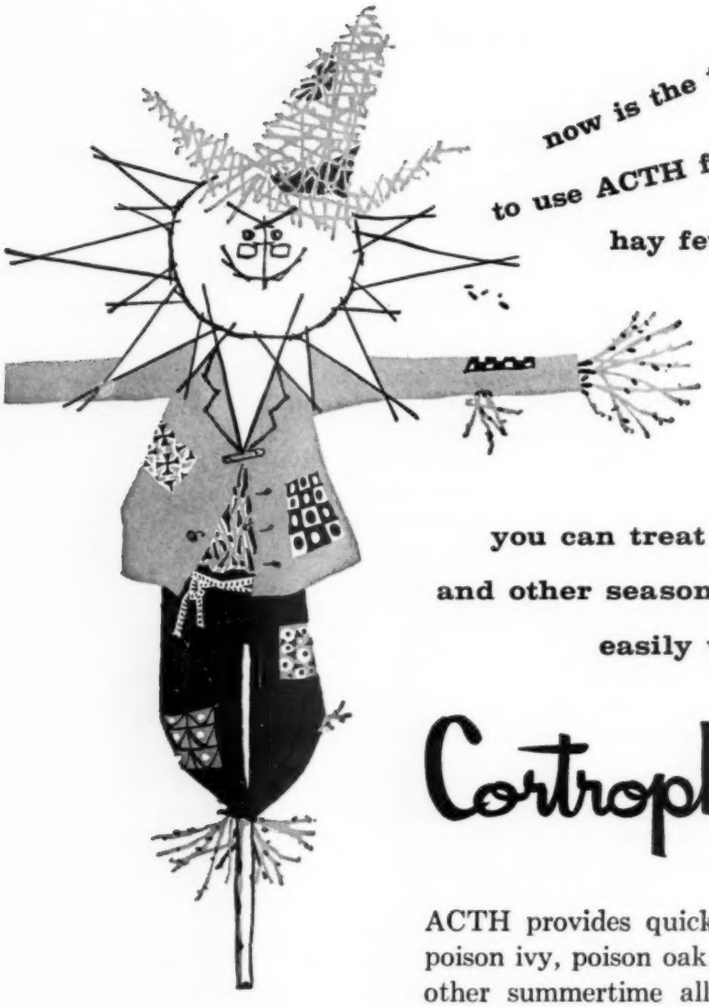

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you can treat HAY FEVER

and other seasonal allergies

easily with

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ORANGE, NEW JERSEY

Printed in U.S.A.

CONTENTS continued–September 1956

VOLUME TWENTY-ONE NUMBER THREE

Advertising Index on 3rd Cover

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"Mediatric" is specially formulated to counteract the adverse influence of declining gonadal function, nutritional inadequacy and emotional instability.

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Nicotinamide (niacinamide)	20 mg.
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Vitamin B_{12} (crystalline)	5 mcg.
Vitamin C (ascorbic acid)	50 mg.

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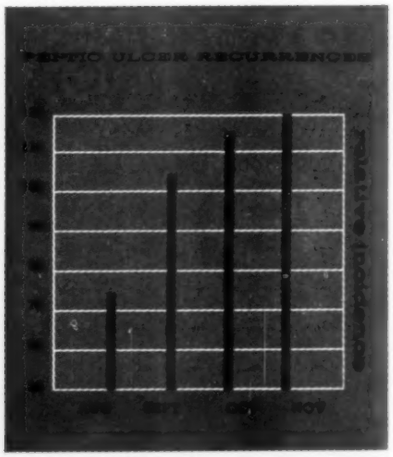

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with singular freedom from bowel and bladder disturbances.[2,3]

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(1) Ivy, A. C.; Grossman, M. I., and Bachrach, W. H.: Peptic Ulcer, Philadelphia, The Blakiston Company, 1950, p. 626. (2) Steigmann, F., and Dolehide, R. A.: Am. J. Digest. Dis. *22*:37, 1955. (3) Riese, J. A.: Am. J. Gastroenterol. *23*:223, 1955.

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Thiamine Mononitrate	6 mg.
Riboflavin	6 mg.
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Calcium Pantothenate	10 mg.
Ascorbic Acid	150 mg.
Liver, Desiccated, N. F.	300 mg. (5 grs.)
Brewer's Yeast, Dried	150 mg. (2½ grs.)

As a dietary supplement: 1 or 2 tablets daily.

For stress, or postoperative convalescence: 2 or more tablets daily.

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Hypotensive action without side effects

RENIR

Reserpine with a safety factor

The desirable hypotensive action of reserpine is often accompanied by distressing side effects. These include nasal congestion, hyperperistalsis, nightmares and mental depression.

Renir, which provides the desired action of reserpine, counterbalanced by the well-known effects of ephedrine, offers the optimum in hypotensive therapy. Untoward reactions are minimized, and tranquilization is maintained.

Investigators state that: "... with reserpine and ephedrine, the untoward effects of each are counteracted and the desirable effects of each are enhanced."[1]

INDICATIONS: In the treatment of mild, moderate and labile hypertension. Also anxiety and tension states; mild to severe neurosis.

SUGGESTED DOSAGE: For hypertension, 1 to 3 tablets daily. As a tranquilizer in mentally disturbed states, 2 to 4 tablets daily.

SUPPLIED: Tablets containing reserpine 0.25 mg., and ephedrine 8.0 mg., in bottles of 100.

CONTRAINDICATIONS: To be used with caution in patients with peptic ulcer, mental depression, cardiac conditions and related disorders.

LITERATURE AND SAMPLES ON REQUEST.

1. *Feinblatt, T.M., Feinblatt, H.M., and Ferguson, E.A.: Rauwolfia-Ephedrine, A Superior Hypotensive-Tranquilizer. In press.*

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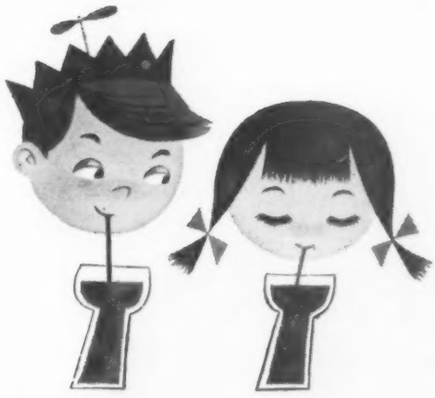

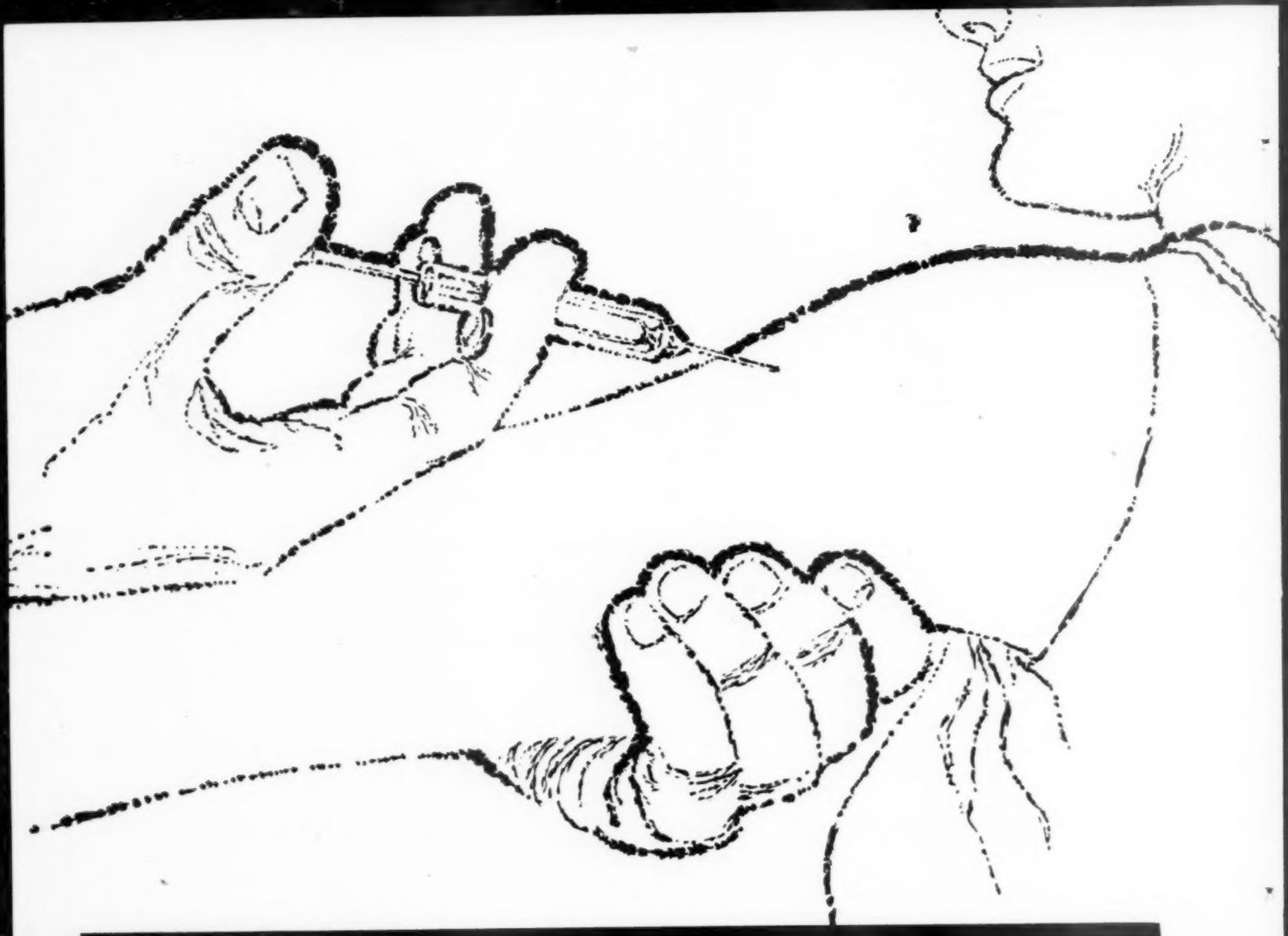

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1. New and Nonofficial Remedies. J.B. Lippincott Co., Philadelphia, 1956, p. 328. 2. Osol, A., and Farrar, G.E., Jr.: The Dispensatory of the United States of America. J.B. Lippincott Co., Philadelphia, 1956, pp. 808-809.

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Patients experience almost immediate relief—calmer days, more restful nights—*beginning the very first day of Nembu-Serpin treatment.*

This faster onset of tranquilizing effect, produced by the synergistic action of Nembutal and reserpine, thus avoids prolonged waiting for a cumulative response to reserpine alone.

And fast-acting Nembu-Serpin makes lower reserpine dosages effective, side effects rare, medication economical. Combines 30 mg. Nembutal (pentobarbital) Calcium and 0.25 mg. reserpine. In bottles of 100 and 500 Filmtabs. Abbott

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New ganglionic blocker lowers blood pressure consistently, predictably, usually with just 2 oral doses per day

Patients with moderate to severe essential hypertension, and even with malignant hypertension, often respond satisfactorily to a ganglionic blocking agent such as Ecolid. It can be used alone or — for maximal control of blood pressure with minimal side effects — in combination with other antihypertensive agents such as Serpasil or Apresoline.

Advantages

Ecolid has the following advantages over other ganglionic blockers:

- long action, usually requires only two oral doses per day
- rapid absorption — promptly reduces systolic and diastolic pressures
- consistent and predictable response — smoother control
- lower dosage required than with other ganglionic blockers
- minimal likelihood of drug tolerance

Clinical observations

In a study of four ganglionic blocking agents, Winsor[1] found that the "most effective agent was SU3088 [Ecolid]...." In another comparative study, Grimson[2] reported: "Results with Ecolid have been definitely more encouraging than those with pentolinium." Patients maintained on Ecolid state that they prefer this ganglionic blocking agent because of greater energy, improved appetite, less difficulty with constipation and fewer tablets to take.[2,3]

For complete information about Ecolid, particularly more details on dosage recommendations, management of undesired effects and precautions, contact your CIBA representative or write to Medical Service Division for booklet entitled "Ecolid — A New Ganglionic Blocker for Hypertension."

References:

1. Winsor, T.: Am. J. M. Sc. *230*:133 (Aug.) 1955. 2. Grimson, K. S.: J.A.M.A. *158*:359 (June 4) 1955. 3. Grimson, K. S., Tarazi, A. K., and Frazer, J. W., Jr.: Circulation *11*:733 (May) 1955. 4. Grimson, K. S., Tarazi, A. K., and Frazer, J. W., Jr.: Angiology *6*:507 (Dec.) 1955. 5. Strawn, J. R., and Moyer, J. H.: Personal communication, 1955. 6. Maxwell, R. D. H., and Howie, T. J. G.: Brit. M. J. *2*:1189 (Nov. 12) 1955.

SUPPLIED: ECOLID Tablets (Rotocotes), 25 mg. (ivory) and 50 mg. (pink).

DOSAGE: Dosage must be adjusted to the individual patient. Below is a typical plan by which treatment may be initiated.

Ambulatory patients		
DAY	A.M.	P.M.
1	25 mg.	—
2	25 mg.	25 mg.
3	50 mg.	25 mg.
4	50 mg.	50 mg.
5	75 mg.	50 mg.
6	75 mg.	75 mg.
7	100 mg.	75 mg.
8	100 mg.	100 mg.

Hospitalized patients		
DAY	A.M.	P.M.
1	50 mg.	—
2	50 mg.	50 mg.
3	100 mg.	50 mg.
4	100 mg.	100 mg.

to optimal response

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chloride
(chlorisondamine chloride CIBA)

REPRESENTATIVE CLINICAL STUDIES OF Ecolid*

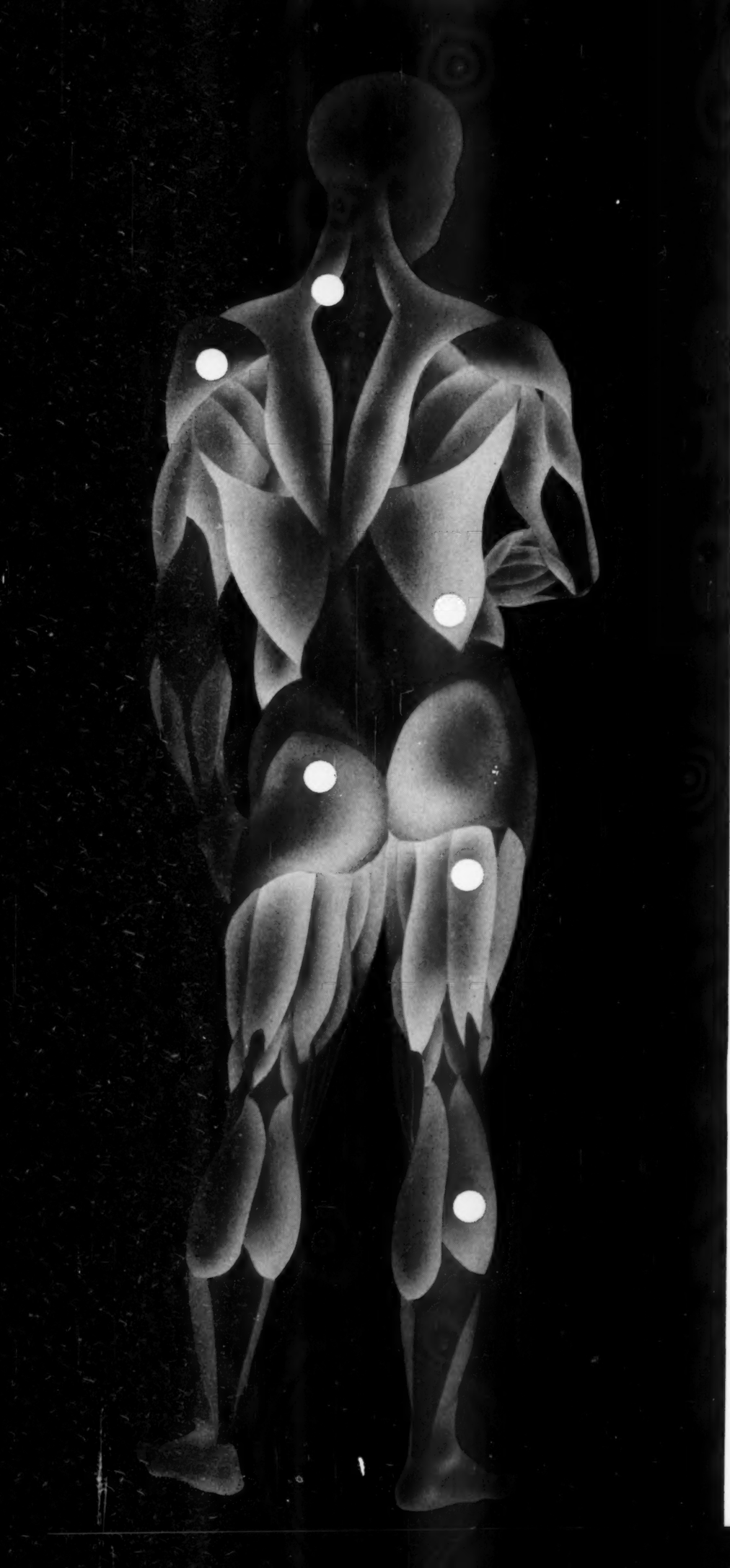
Number of Patients	Initial Oral Dosage	Responses	Duration of Action	References
59 (Total Series)	75 to 300 mg. daily	Compared with other ganglionic blockers, small doses of Ecolid were employed and greater hypotensive effect was obtained. Rapid absorption and long duration of hypotensive action.	Postural hypotension lasted 13.4 hours in 5 "test" patients receiving doses of 150 mg.	1
20	50 to 200 mg. daily	Blood pressure in 20 well controlled; reductions lasted twice as long as those induced by pentolinium. Each of 10 patients with previous experience with hexamethonium preferred Ecolid. Less difficulty with constipation; appetite improved; greater energy.	**	2
18	50 to 100 mg. daily	Hypertension in 18 well controlled. Supine blood pressure reduced without tachycardia. Constipation occurred infrequently.	Supine blood pressure lowered for 12 hours or more with single oral doses of 50 to 100 mg.	3,4
44	50 mg. daily	35 responded well; 14 of these became normotensive. All patients received reserpine as base therapy.	**	5
12	25 to 200 mg. daily	Blood pressure of all 12 satisfactorily controlled. Systolic blood pressure lowered average of 76 mm. Diastolic blood pressure lowered average of 42 mm.	**	6

*To date, a total of 63 investigators have reported on the use of Ecolid in more than 500 patients. They were practically unanimous in the opinion that Ecolid was highly effective. Nearly all commented on the prolonged duration of action—about 8 to 12 hours—which permitted a twice daily dosage schedule in most cases.

**Information not available.

SUMMIT, N. J.

2/2251M



WHEREVER

SKELETAL

MUSCLE

SPASM

OCCURS...

flexin*

(Zoxazolamine,† McNeil)

orally effective muscle relaxant

safe:

"No irreversible side-effects occurred."[1]

well-tolerated:

"The toxic reactions for the most part were easily controlled...."[1]

effective spasmolytic:

"This preliminary report of 100 patients indicates an 85% over-all effectiveness."[1]

Available in yellow scored tablets, 250 mg.

1. Smith, R. T.; Kron, K. M.; Peak, W. P., and Hermann, I. F.: J.A.M.A. *160*:745 (Mar. 3) 1956.

*T.M.
†U.S. Patent Pending

McNEIL Laboratories, Inc. • Philadelphia 32, Pa.

for preventing and treating upper respiratory infections

new!

Achrocidin*

TETRACYCLINE-ANTIHISTAMINE-ANALGESIC COMPOUND

ACHROCIDIN provides in one tablet all the drugs which are often prescribed separately for the prevention and treatment of cold complications —conditions such as otitis, adenitis, sinusitis, and others.

This comprehensive formula 1) provides potent therapeutic and prophylactic action against a wide variety of infective organisms, 2) relieves pain and discomfort, 3) depresses fever, 4) alleviates nasal congestion.

Available on prescription only

Each tablet contains:

ACHROMYCIN® Tetracycline	125 mg.
Phenacetin	120 mg.
Caffeine	30 mg.
Salicylamide	150 mg.
Chlorothen Citrate	25 mg.

Bottle of 24 tablets.

Average adult dose: 2 tablets, 4 times daily

LEDERLE LABORATORIES DIVISION, AMERICAN CYANAMID COMPANY, PEARL RIVER, NEW YORK

*TRADE-MARK

Lederle

For patients pursued by their own emotions —

Noludar 'Roche' will help solve the problem. Not a barbiturate, not habit forming, 50 mg t.i.d. provides daytime sedation without somnolence, while 200 mg h.s. induces a sound night's sleep without hangover.

Noludar tablets, 50 and 200 mg; elixir, 50 mg per teaspoon.

Hoffmann - La Roche Inc
Nutley 10, New Jersey

Noludar®
brand of methyprylon

DRY, SCALY SKIN
DETERGENT RASH
CHAFING
PRICKLY HEAT
SUNBURN
'DISHPAN' HANDS
DIAPER RASH
SIMPLE ECZEMA

Superficial skin complaints usually respond dramatically to TASHAN CREAM 'Roche.' Antiprurient, soothing, and healing--contains vitamins A, D, E, and d-Panthenol, in a cosmetically pleasing water-soluble base which fastidious patients will enjoy using. Hoffmann-La Roche Inc, Nutley, N. J.

TASHAN T.M.

after severe illness . . . the water-soluble vitamins

Combex® with Vitamin C Kapseals®

After severe injury or illness, vitamin deficiencies are most frequently due to lack of water-soluble vitamins.* By prescribing COMBEX WITH VITAMIN C KAPSEALS you provide convalescent patients with dependable dosage of several factors of the vitamin B-complex and of vitamin C—both in a single, convenient KAPSEAL.

Other members of the COMBEX family include:

COMBEX KAPSEALS
COMBEX PARENTERAL } —factors of vitamin B-complex

THERA-COMBEX® KAPSEALS—Therapeutic doses of factors of vitamin B-complex and of vitamin C with Taka-Diastase®

TAKA-COMBEX® KAPSEALS—Factors of vitamin B-complex, C, and Taka-Diastase

TAKA-COMBEX ELIXIR—Factors of vitamin B-complex and Taka-Diastase

*Pollack, H., and Halpern, S. L.: Therapeutic Nutrition, Washington, D. C., National Academy of Sciences—National Research Council, 1952, p. 21.

50054

MEDICAMENTA VERA

PARKE, DAVIS & COMPANY DETROIT, MICHIGAN

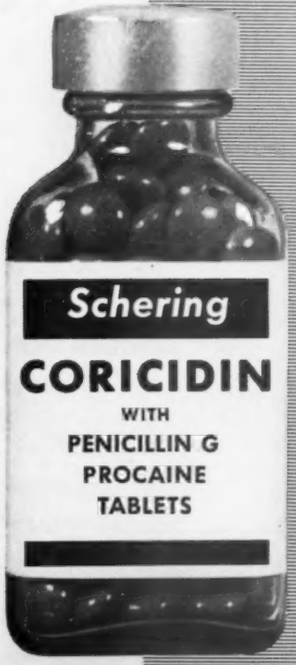
restrict cold complications . . .

relieve the patient

CORICIDIN with Penicillin

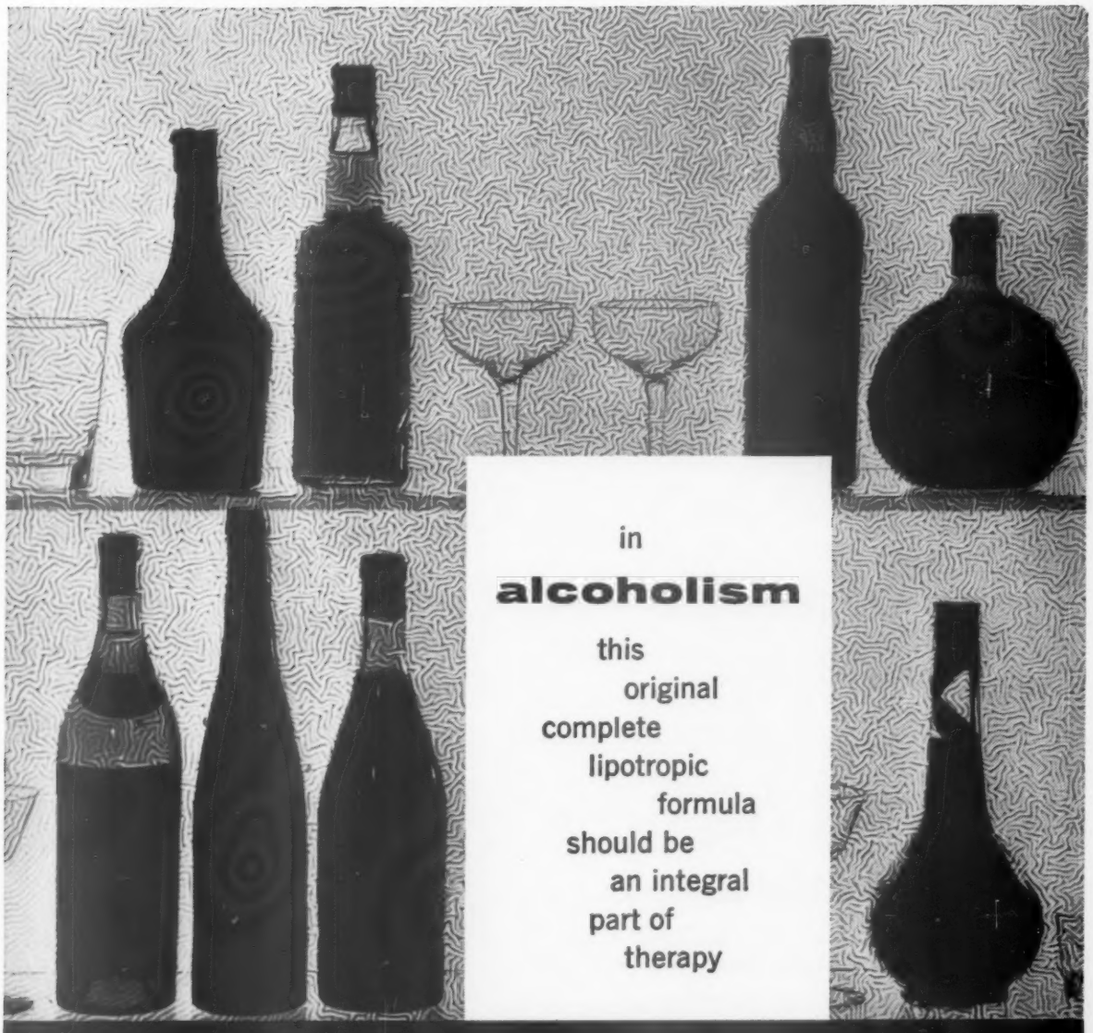
(150,000 units Penicillin G Procaine) **TABLETS**

especially in severe, persistent colds
unsurpassed symptomatic relief simultaneously with antibacterial protection...to ward off common complications such as bronchitis and laryngitis...to help shorten recovery period.



CORICIDIN,® brand of analgesic-antipyretic.

CN-J-14106



in

alcoholism

this
original
complete
lipotropic
formula
should be
an integral
part of
therapy

methischol®

methionine • vitamin B_{12} • choline • inositol • liver

to reverse and prevent further liver damage and hepatic cirrhosis (so common in alcoholism)

METHISCHOL helps to increase phospholipid turnover, reduce fatty deposits, lessen tendency to fibrosis and aids in regeneration of new liver cells.

as a protective aid against atherosclerosis and coronary impairment

METHISCHOL helps reduce elevated cholesterol levels and helps lower chylomicron-lipomicron ratios towards normal.

capsules bottles of 100, 250, 500 and 1000.
syrup bottles of 16 ounces and 1 gallon.

for samples and detailed literature write

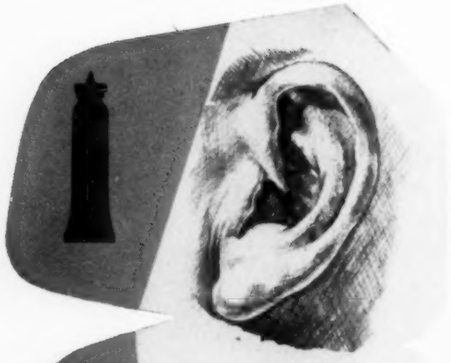
u. s. vitamin corporation

(Arlington-Funk Laboratories, division)
250 East 43rd Street, New York 17, N.Y.

for superficial bacterial infections of the skin and external ear

Spectrocin Ointment

Squibb Neomycin-Gramicidin in Plastibase*
15 and 30 gram tubes



for superficial bacterial infections of the eye

Spectrocin Ophthalmic Ointment

Squibb Neomycin-Gramicidin in Plastibase*
3.6 gram ophthalmic tubes

for symptomatic relief of minor throat irritations

Spectrocin-T

Squibb Neomycin-Gramicidin-Benzocaine Troches
boxes of 10 and bottles of 48

The organisms responsible for most superficial bacterial infections are highly susceptible to neomycin; those which are only slightly susceptible or resistant to neomycin are usually susceptible to gramicidin.

Neomycin is rarely administered systemically, and gramicidin never. With Spectrocin (Squibb Neomycin-Gramicidin), therefore, there is no danger of sensitizing patients to antibiotics generally used systemically for serious infections.

SQUIBB *Squibb Quality—the Priceless Ingredient*

*(SQUIBB OLEAGINOUS OINTMENT BASE)

'SPECTROCIN'® AND 'PLASTIBASE' ARE SQUIBB TRADEMARKS

SP-56

Modern medical research instruments

THESE seven-ton bulldozers are truly research tools, for they are taking part in an exhaustive program for the study and revision of accepted methods of medical instrument design and manufacture.

Yes, Sanborn Company is on the move! The instruments above are levelling off small mountains of earth and rock in preparation for a new and modern Sanborn plant near Boston, Mass.

Completion of the structure late this year will mean vastly improved facilities for research, manufacturing and other operations. This will directly and immediately benefit not only the work Sanborn does, but also the people who use Sanborn instruments. It will make possible more rapid development of new instruments . . . faster production, delivery and service . . . and increased opportunity for a larger number of people to apply their skills to the problems of modern instrument design and manufacture.

Sanborn Company, Cambridge 39, Massachusetts

Scale model of new Sanborn plant just off Route 128 in Waltham, Mass.

At Last...

SIMPLIFIED NEBULIZATION THERAPY for Asthma

Only

MEDIHALER®

Provides Measured, Uniform Dosage Inhalation Therapy, Trouble-Free, Promptly Effective

- True Nebulization—80 per cent of particles measure from 0.5 to 4 microns radius—insuring effective penetration of respiratory tract.
- Medication, in leakproof, spillproof bottle, cannot deteriorate by exposure to light or oxidation. Each 10 cc. bottle provides 200 inhalations.
- Medihaler Oral Adapter is inexpensive, unbreakable. No breakage of costly glass nebulizers.
- Aerosol dose released is always the same, does not depend on patient strength or on amount in bottle. One or occasionally two inhalations provides relief for most patients. Notably safe for use with children.

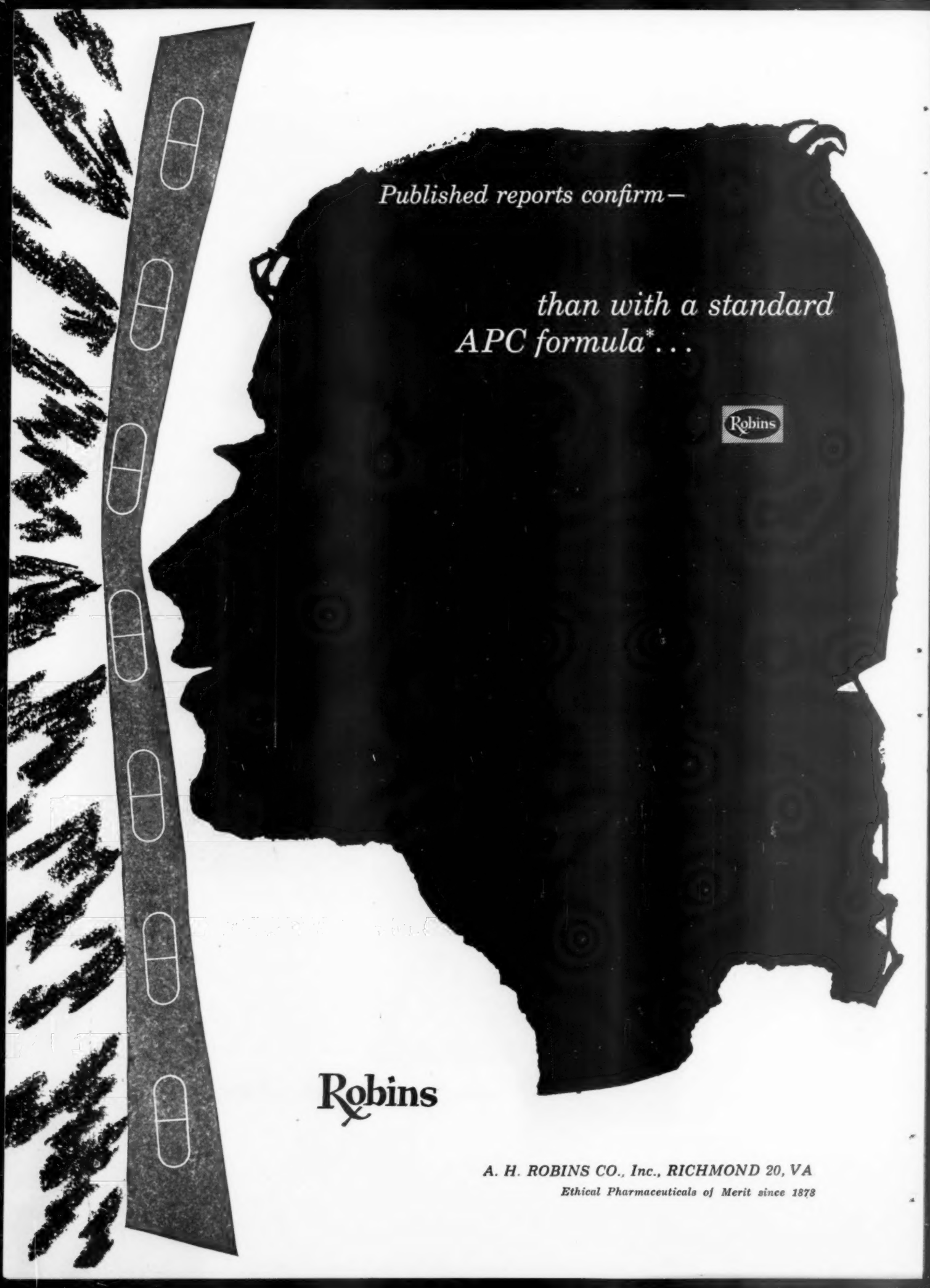
***MEDIHALER-EPI™**
0.5% solution of epinephrine U.S.P.

***MEDIHALER-ISO™**
0.25% solution of isoproterenol HCl U.S.P.

In your first prescription for the patient be sure to write for medication (whichever you choose) AND the Medihaler Oral Adapter (packaged and sold separately), since medications cannot be used without Adapter. For refills, write Rx for medication only.

*The Medihaler principle of effective antiasthmatic therapy offers your favorite bronchodilators in special Medihaler aerosol form.

Another First from **Riker** LOS ANGELES



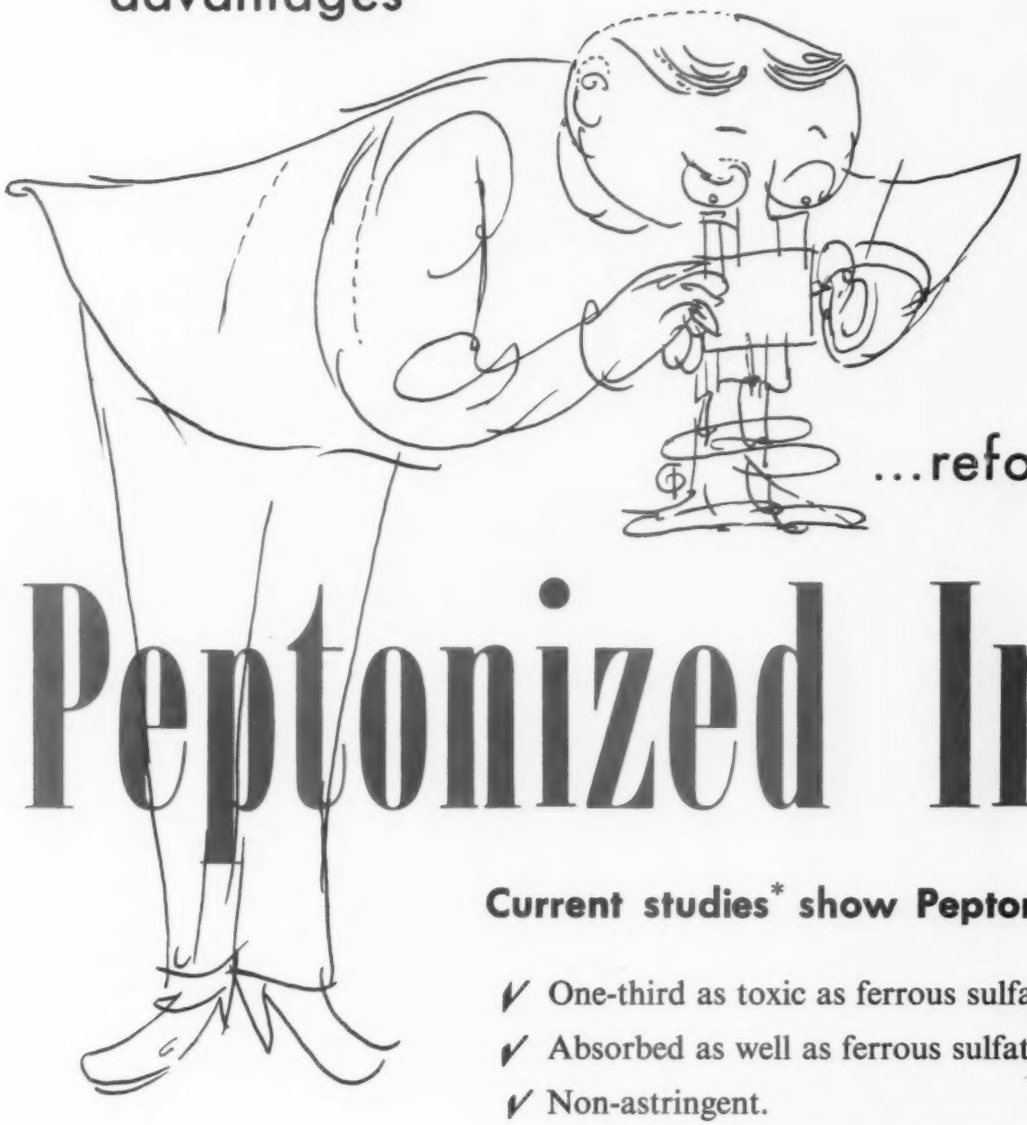
A. H. ROBINS CO., Inc., RICHMOND 20, VA

Ethical Pharmaceuticals of Merit since 1878

for assured

therapeutic

advantages



...refocus on...

Peptonized Iron

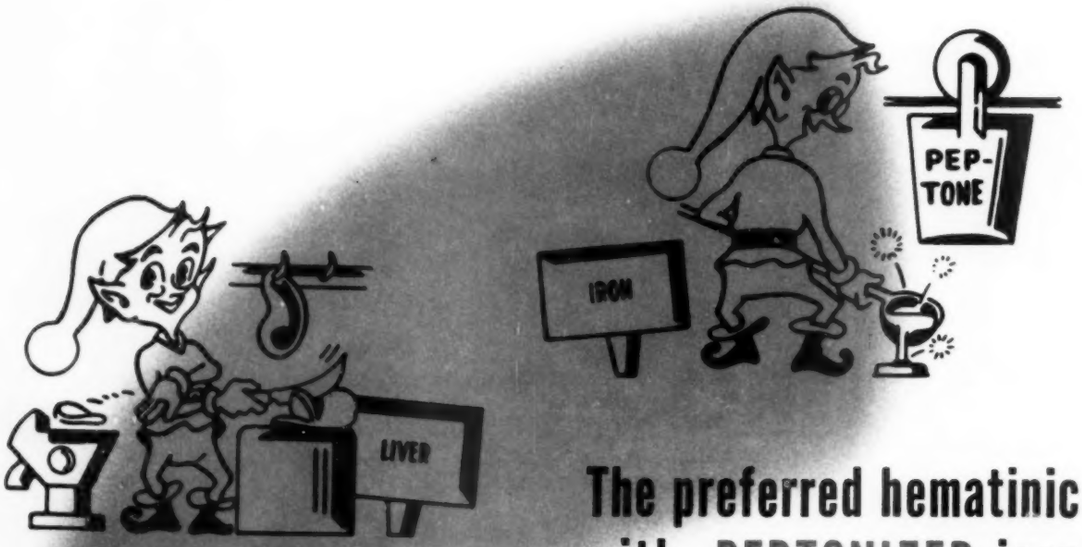
Current studies* show Peptonized Iron—

- ✓ One-third as toxic as ferrous sulfate.
- ✓ Absorbed as well as ferrous sulfate.
- ✓ Non-astringent.
- ✓ Free from tendencies to disturb digestion. (*One-tenth as irritating to the gastric mucosa as ferrous sulfate.*)
- ✓ More effective in iron-deficient anemias.

LIVITAMIN® with Peptonized Iron

*Keith, J. H.: Utilization and Toxicity of Peptonized Iron and Ferrous Sulfate, Read before the American Association for the Advancement of Science, Zoological Section, Atlanta, Georgia, December, 1955.

THE S. E. MASSENGILL COMPANY Bristol, Tennessee • New York • Kansas City • San Francisco



The preferred hematinic with PEPTONIZED iron

LIVITAMIN®

Peptonized iron is virtually predigested. It is absorbed as well as ferrous sulfate, and is one-tenth as irritating to the gastric mucosa. Anemias refractory to other forms of iron will often respond promptly to Livitamin therapy.

The Livitamin formula, containing the B complex, provides integrated therapy to correct the blood picture, and to improve appetite and digestion.

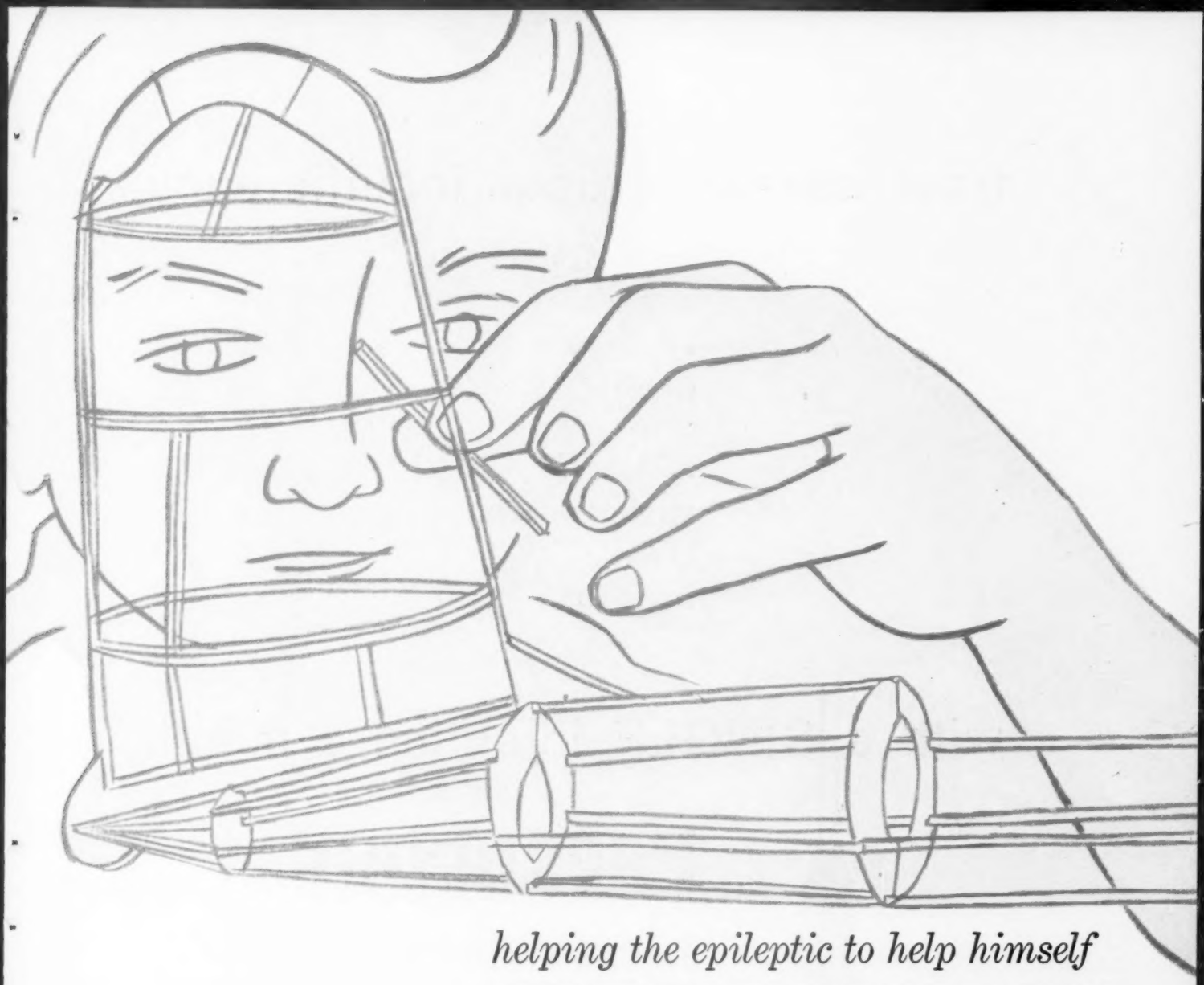
Each fluidounce contains:

Iron peptonized (Equiv. in elemental iron to 71 mg.)	420 mg.
Manganese citrate, soluble	158 mg.
Thiamine hydrochloride	10 mg.
Riboflavin	10 mg.
Vitamin B_{12} (crystalline)	20 mcg.
Niacinamide	50 mg.
Pyridoxine hydrochloride	1 mg.
Pantothenic acid	5 mg.
Liver fraction 1	2 Gm.
Rice bran extract	1 Gm.
Inositol	30 mg.
Choline	60 mg.

THE S. E. MASSENGILL COMPANY

Bristol, Tennessee

New York Kansas City San Francisco



helping the epileptic to help himself

MILONTIN®

Kapseals® and Suspension

(phensuximide, Parke-Davis)

for patients with petit mal epilepsy

A drug of choice in initiating treatment and, after five years of study, found least toxic of all effective drugs.[1] Often effective in patients refractory to other therapy... and often of definite value in some patients with psychomotor epilepsy.

MILONTIN Kapseals, 0.5 Gm., bottles of 100 and 1,000; also available as MILONTIN Suspension (250 mg. per 4 cc.) in 16-ounce bottles.

DILANTIN® SODIUM

(diphenylhydantoin sodium, Parke-Davis)

for patients with grand mal and psychomotor seizures

Alone or in combination, DILANTIN continues as an anticonvulsant of choice... with time-tested advantages of greater safety and lack of hypnotic activity.[2,3]

DILANTIN Sodium is supplied in a variety of forms—including Kapseals of 0.03 Gm. (½ gr.) and 0.1 Gm. (1½ gr.), bottles of 100 and 1,000.

For patients with mixed grand mal—petit mal epilepsy, MILONTIN may be used in combination with DILANTIN Sodium or with DILANTIN Sodium with Phenobarbital.

(1) Zimmerman, F. T.: *New York J. Med.* 55:2338, 1955. (2) Drake, F. R.: *Am. J. M. Sc.* 230:98, 1955. (3) Levy, L., & Shanbrom, E.: *Arch. Int. Med.* 97:599, 1956.

MEDICAMENTA VERA

PARKE, DAVIS & COMPANY • DETROIT 32, MICHIGAN

50025

treat adrenocortical insufficiency

without the
difficulties
of
implants

without the
unpleasantness
of daily
injections

with a SINGLE injection a month

Percorten®

trimethylacetate

(desoxycorticosterone trimethylacetate CIBA)

Percorten trimethylacetate, developed after more than 3 years of research by CIBA, provides expedient, lasting hormonal support for the patient with adrenocortical hypofunction . . . avoiding both the uncertainty of surgical implantation and the vexation of daily injections. With once-a-month therapy, a single injection will produce prolonged activity without acute signs of overdosage.[1]

Multiple-dose Vials, 4 ml., containing 25 mg. Percorten trimethylacetate per ml. as an aqueous microcrystalline suspension for intramuscular use only.

1. Frawley, T. F., and Forsham, P. H.: J. Clin. Endocrinol. *11*:772 (July) 1951.

CIBA
SUMMIT, N. J.

8/[illegible]

A penny saved is a penny ------

Something missing? Sure—that important last word!

When you prescribe prenatal capsules, the word to remember is *Lederle*. Write it, and assure your patient the genuine Lederle formula!

PRENATAL CAPSULES LEDERLE

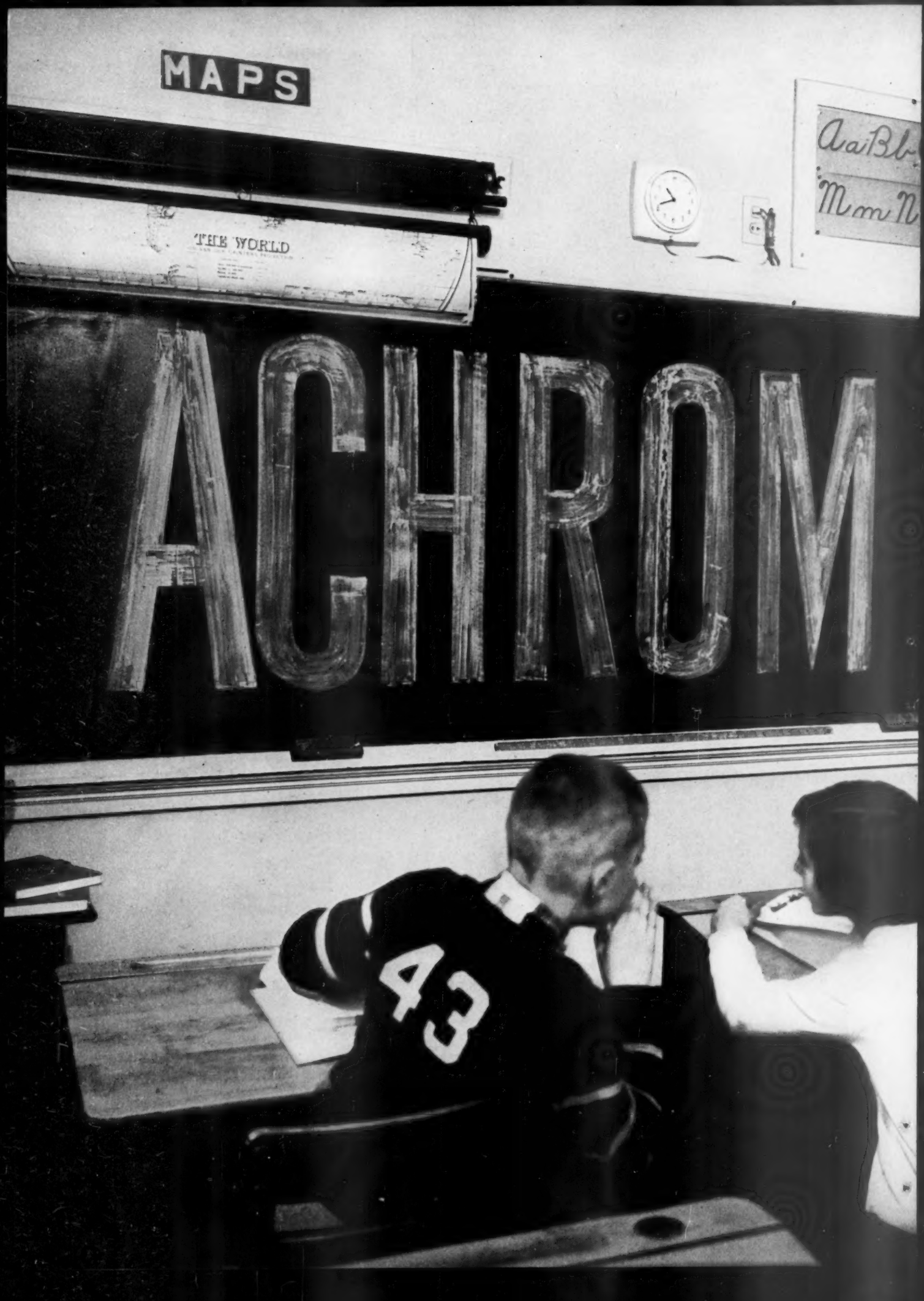
Dosage: 1 to 3 capsules daily, throughout pregnancy and lactation.

Each capsule contains:

Vitamin A	2000 U.S.P. Units	Folic Acid	1 mg.
Vitamin D	400 U.S.P. Units	Calcium (in $CaHPO_4$)	250 mg.
Thiamine Mononitrate (B_1)	2 mg.	Phosphorus (in $CaHPO_4$)	190 mg.
Riboflavin (B_2)	2 mg.	Dicalcium Phosphate Anhydrous ($CaHPO_4$)	869 mg.
Niacinamide	7 mg.	Iron (in $FeSO_4$)	6 mg.
Vitamin B_{12}	1 mcgm.	Ferrous Sulfate Exsiccated	20 mg.
Vitamin K (Menadione)	0.5 mg.	Manganese (in $MnSO_4$)	0.12 mg.
Ascorbic Acid (C)	35 mg.		

dry-filled sealed capsules—a Lederle exclusive! More rapidly and completely absorbed. No oils, no paste . . . no aftertaste.

LEDERLE LABORATORIES DIVISION AMERICAN Cyanamid COMPANY PEARL RIVER, NEW YORK Lederle


MAPS
THE WORLD
ACHROM
43

ACHROMYCIN

Tetracycline *Lederle*

in the treatment of

respiratory infections

January and his associates[1] have written on the use of tetracycline (ACHROMYCIN) to treat 118 patients having various infections, most of them respiratory, including acute pharyngitis and tonsillitis, otitis media, sinusitis, acute and chronic bronchitis, asthmatic bronchitis, bronchiectasis, bronchial pneumonia, and lobar pneumonia. Response was judged good or satisfactory in more than 84% of the total cases.

Each month there are more and more reports like this in the literature, documenting the great worth and versatility of ACHROMYCIN. This antibiotic is unsurpassed in range of effectiveness. It provides rapid penetration, prompt control. Side effects, if any, are usually negligible.

No matter what your field or specialty, ACHROMYCIN can be of service to you. For your convenience and the patient's comfort, Lederle offers a *full* line of dosage forms, including

ACHROMYCIN SF

ACHROMYCIN with STRESS FORMULA VITAMINS. Attacks the infection—defends the patient—hastens normal recovery. For severe or prolonged illness. Stress formula as suggested by the National Research Council. Offered in Capsules of 250 mg. and in an Oral Suspension, 125 mg. per 5 cc. teaspoonful.

For more rapid and complete absorption. Offered only by Lederle!

dry-filled sealed capsules

[1]January, H. L. et al: Clinical experience with tetracycline. *Antibiotics Annual* 1954-55, p. 625.

Lederle

LEDERLE LABORATORIES DIVISION
AMERICAN CYANAMID COMPANY
PEARL RIVER, NEW YORK

®REG. U. S. PAT. OFF.

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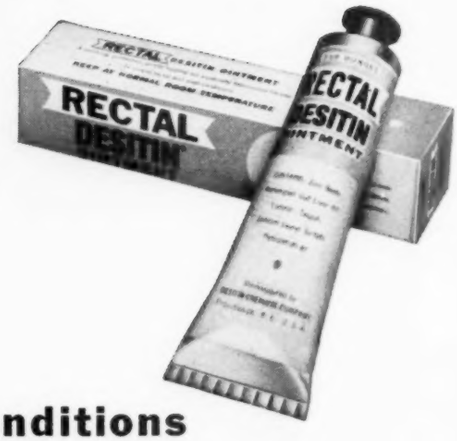
RECTAL DESITIN® OINTMENT



NEW and specially formulated for more effective and sustained relief in

anorectal conditions

RECTAL DESITIN OINTMENT affords unusually effective relief from pain, irritation, inflammation, itching, congestion, and discomfort in non-surgical hemorrhoids, anorectal irritation, pruritus, uncomplicated fissures, proctitis, inflammatory cryptitis, papillitis and perianal dermatitis.

Outstanding Advantages: (1) a special tacky consistency for prolonged efficacy. (2) a unique wetting agent for intimate, thorough coverage. (3) Norwegian cod liver oil to stimulate healing.

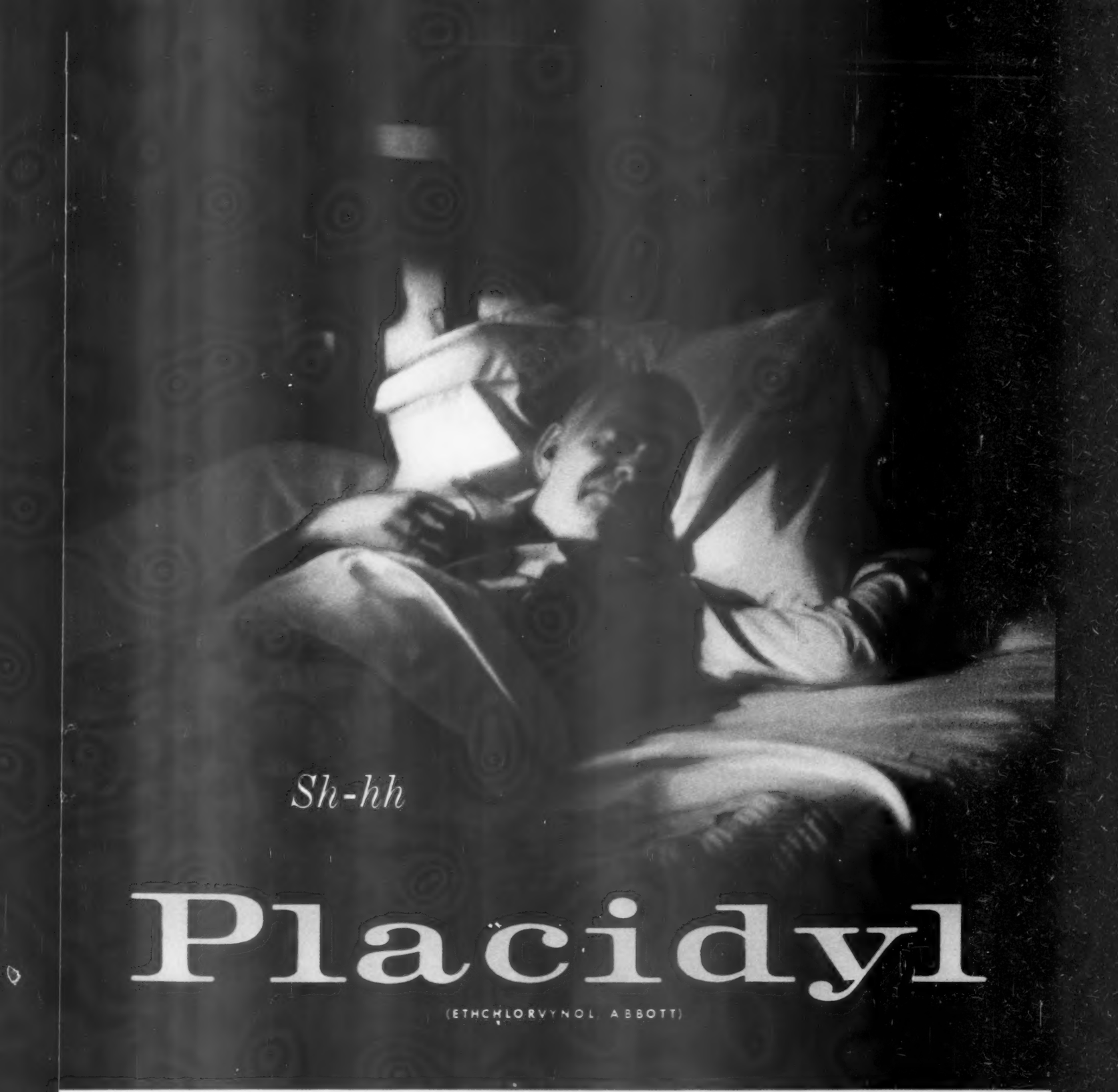
Formula: RECTAL DESITIN OINTMENT contains high grade Norwegian cod liver oil, zinc oxide, lanolin, talcum, sodium lauryl sulfate, petrolatum q.s. Does *not* contain local anesthetics, narcotics, or "caine" drugs which might mask serious anorectal disorders.

Available on your prescription in tubes of 1½ oz., with a safe, flexible applicator.

Liberal **sample** supply on request.

DESITIN CHEMICAL COMPANY
Providence, R. I.

New RECTAL DESITIN OINTMENT is not to be confused with regular DESITIN OINTMENT.



nudges your patient to sleep

One 500-mg. capsule of this new nonbarbiturate gently relieves ordinary nervous insomnia. Excellent in presence of mild anxiety or unrest, chronic disease, old age, and small hour waking. Abbott

609021

Selsun®

...most effective treatment known for seborrheic dermatitis of the scalp and dandruff...

Your prescription for Selsun assures patients lasting relief from itching, scaling, burning scalps. And patients appreciate the ease of using Selsun: applies like a shampoo, rinses out easily, leaves both hair and scalp clean. Selsun Suspension completely controls 81-87% of seborrheic dermatitis, 92-95% of dandruff cases. Each 4-fluidounce bottle carries full directions.

Abbott®

®Selenium Sulfide, Abbott

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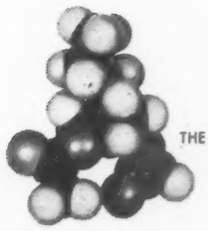


THE MILTOWN MOLECULE

A tranquilizer well suited for prolonged therapy

NO ORGANIC CONTRAINDICATIONS

reported to date

- well tolerated, non-addictive, essentially non-toxic
- no blood dyscrasias, liver toxicity, Parkinson-like syndrome or nasal stuffiness
- chemically unrelated to chlorpromazine or reserpine
- does not produce significant depression
- orally effective within 30 minutes for a period of 6 hours

Indications: anxiety and tension states, muscle spasm.

Miltown®

THE ORIGINAL MEPROBAMATE

DISCOVERED AND INTRODUCED by Wallace Laboratories, New Brunswick, N. J.

2-methyl-2-*n*-propyl-1,3-propanediol dicarbamate—U. S. Patent 2,724,720

SUPPLIED: 400 mg. scored tablets. Usual dose: 1 or 2 tablets t.i.d.

Literature and Samples Available on Request

Desbutal®

(DESOXYN® plus NEMBUTAL®)

PHOTOGRAPH BY JIM BROWN

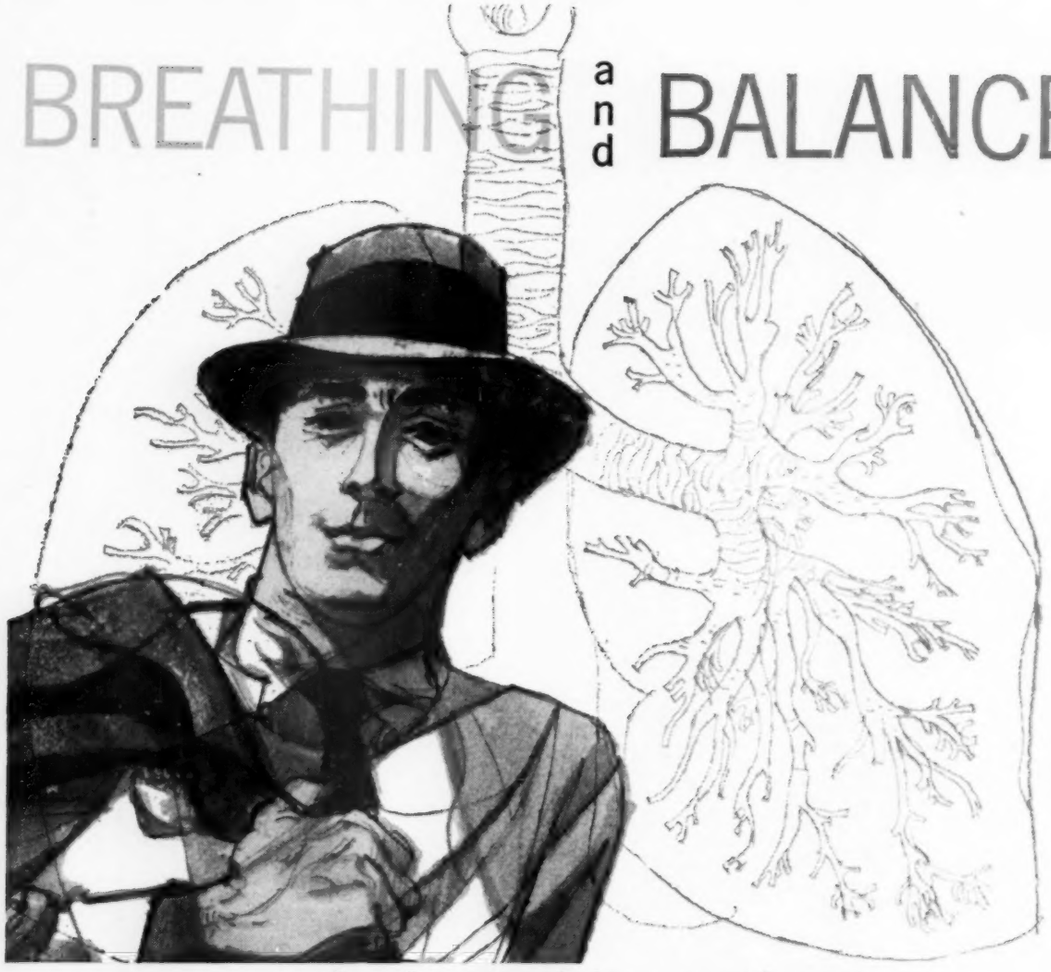
509226

to dispel anxiety and depression

One capsule represents 5 mg. DESOXYN (Methamphetamine, Abbott) Hydrochloride and 30 mg. NEMBUTAL (Pentobarbital, Abbott) Sodium.

Abbott

BREATHING and BALANCE



in bronchial asthma

Sterane®

brand of prednisolone

one of "the best therapeutic agents now available"*

provides restoration of breathing capacity — Relief of symptoms [bronchospasm, cough, wheezing, dyspnea] is maintained for long periods with relatively small doses.*

minimal effect on electrolyte balance — "in therapeutically effective doses . . . there is usually no sodium or fluid retention or potassium loss."* Lack of edema and undesirable weight gain permits more effective therapy particularly for those with cardiac complications.

Supplied: White, 5 mg. oral tablets, bottles of 20 and 100. Pink, 1 mg. oral tablets, bottles of 100. Both are deep-scored.

*Schwartz, E.: New York J. Med. 56:570, 1956.

PFIZER LABORATORIES, Brooklyn 6, New York
Division, Chas. Pfizer & Co., Inc.

for those with

PARKINSONISM

Smoother activity

and

brighter expression

with 'KEMADRIN'®*

- reduces rigidity and tremor.
- seldom causes dryness of the mouth, blurring of vision or excitation.

***'KEMADRIN'** brand Procyclidine Hydrochloride Tablet of 5 mg., scored. Bottles of 100 and 1,000.

Literature available on request.

BURROUGHS WELLCOME & CO. (U. S. A.) INC., Tuckahoe, N.Y.

H+ $\overline{OH}$

Superior antacid action and...

"For palatability, many patients prefer Maalox"[1]

MAALOX®, an efficient antacid suspension of magnesium-aluminum hydroxide gel, is smooth-textured, and always pleasant to take. MAALOX was tested by thousands of hospital outpatients, who preferred it to other antacids. Indeed, *high patient acceptability* (without danger of constipation) is one of the outstanding advantages of MAALOX therapy.[2]

As to chemistry: MAALOX has more acid-binding capacity than aluminum hydroxide gel, and maintains its antacid effect twice as long.[3]

Supplied: *Suspension*, bottles of 12 fluidounces. *Tablets*, bottles of 100. Samples sent promptly on request.

1. Kramer, P.: Med. Clin. North America, 39:1381, Sept. 1955.
2. Morrison, Samuel: Am. J. Gastroenterology 22:309 (1954).
3. Rossett, N. E., Rice, M. L., Jr., Gastroenterology 26:490 (1954).

For Pain
try Ascriptin Tablets
(Aspirin buffered with Maalox)
- Doubles blood salicylate level
- Action more prolonged
- High gastric tolerance level
- Clinically proved.

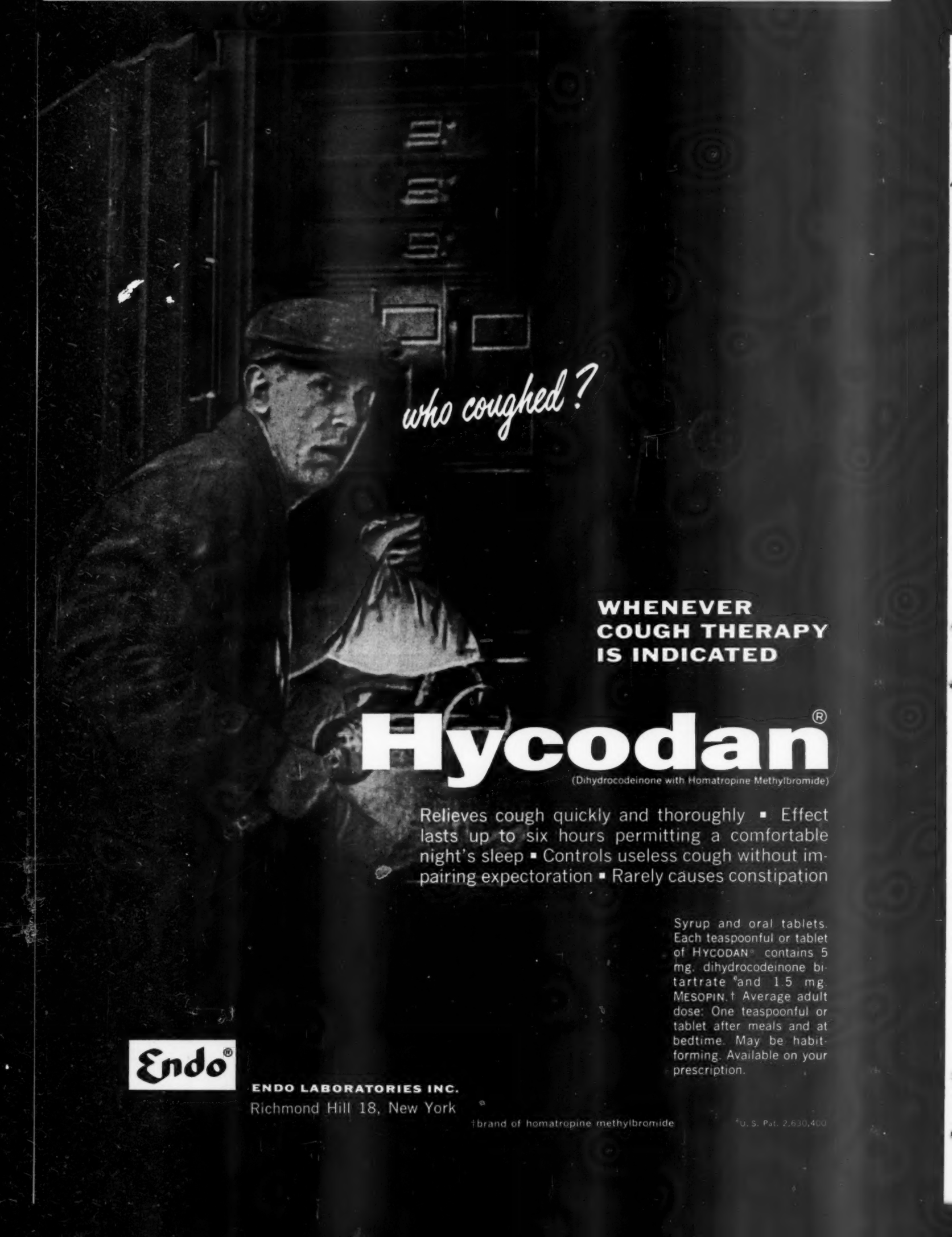
Samples on request.

Ascriptin

Maalox®

"... better suited for antacid therapy"[2]

WILLIAM H. RORER, Inc. RORER PHILADELPHIA, PA.



WHENEVER COUGH THERAPY IS INDICATED

Hycodan®

(Dihydrocodeinone with Homatropine Methylbromide)

Relieves cough quickly and thoroughly ▪ Effect lasts up to six hours permitting a comfortable night's sleep ▪ Controls useless cough without impairing expectoration ▪ Rarely causes constipation

Syrup and oral tablets. Each teaspoonful or tablet of HYCODAN* contains 5 mg. dihydrocodeinone bitartrate *and 1.5 mg. MESOPIN.† Average adult dose: One teaspoonful or tablet after meals and at bedtime. May be habit-forming. Available on your prescription.

Endo®

ENDO LABORATORIES INC.
Richmond Hill 18, New York

†brand of homatropine methylbromide

*U. S. Pat. 2,630,400

TANGIBLE RELIEF IN PREMENSTRUAL TENSION

another indication for Diamox*

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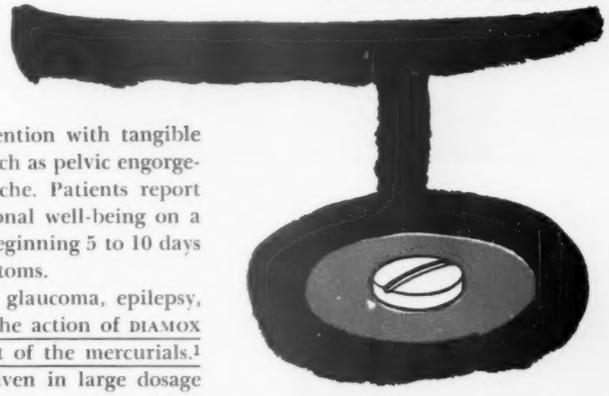

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1 Krantz, J. C. and Carr, C. J.: The Pharmacologic Principles of Medical Practice. Ed. 3. The Williams & Wilkins Co., Baltimore, 1954, p. 1014.

2 Goodman, L. S. and Gilman, A.: The Pharmacological Basis of Therapeutics. Ed. 2. The Macmillan Co., New York, 1955, p. 856.

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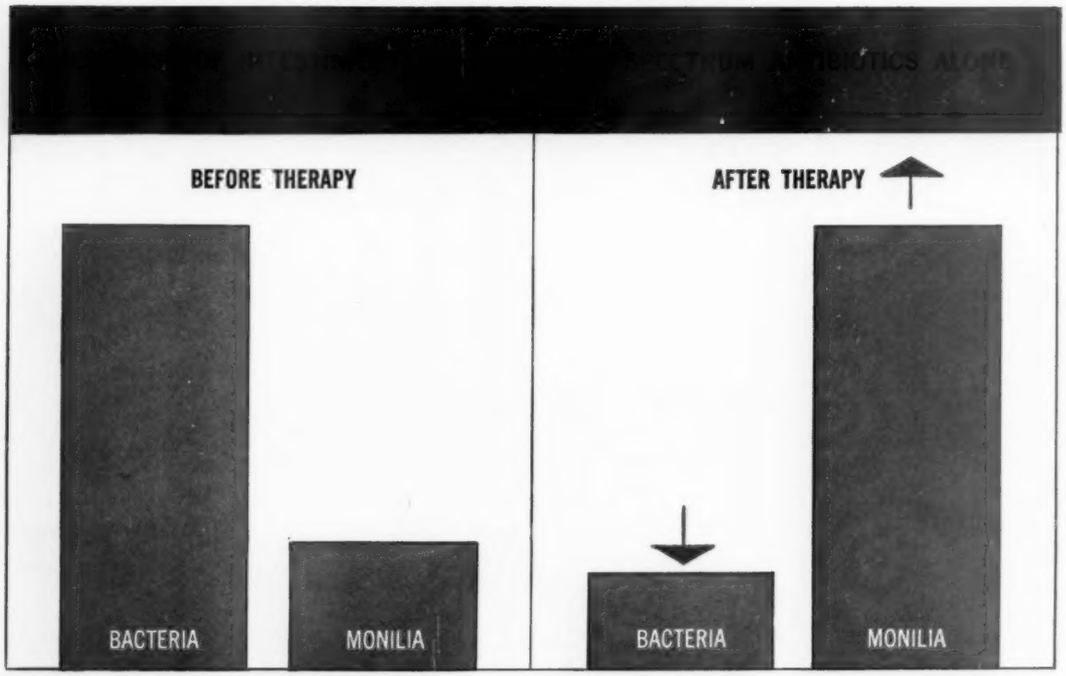

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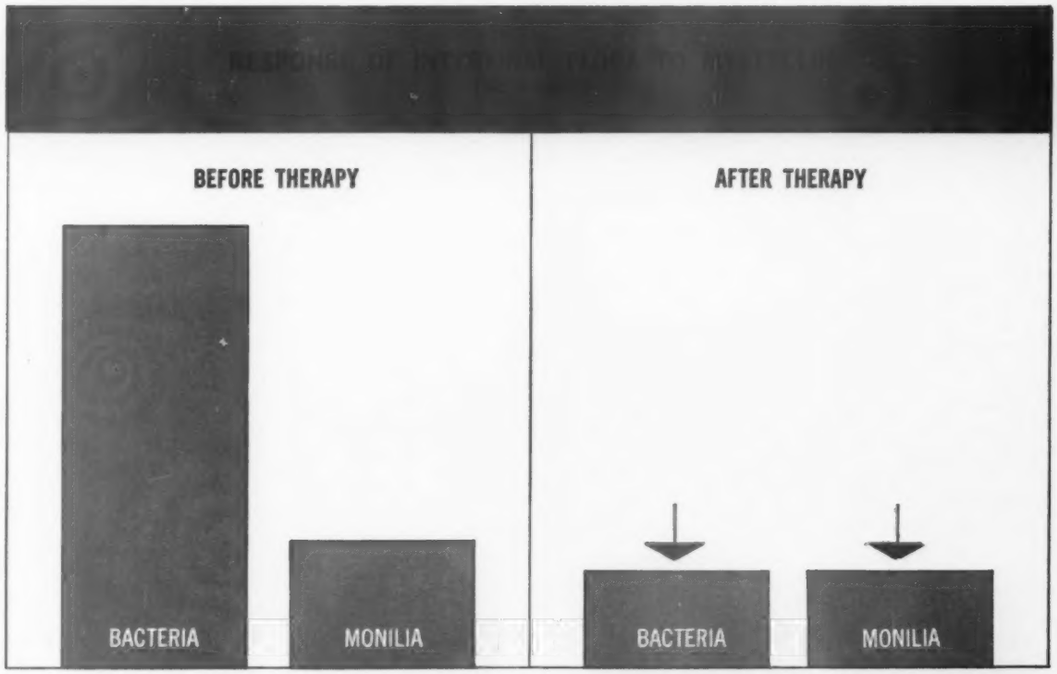

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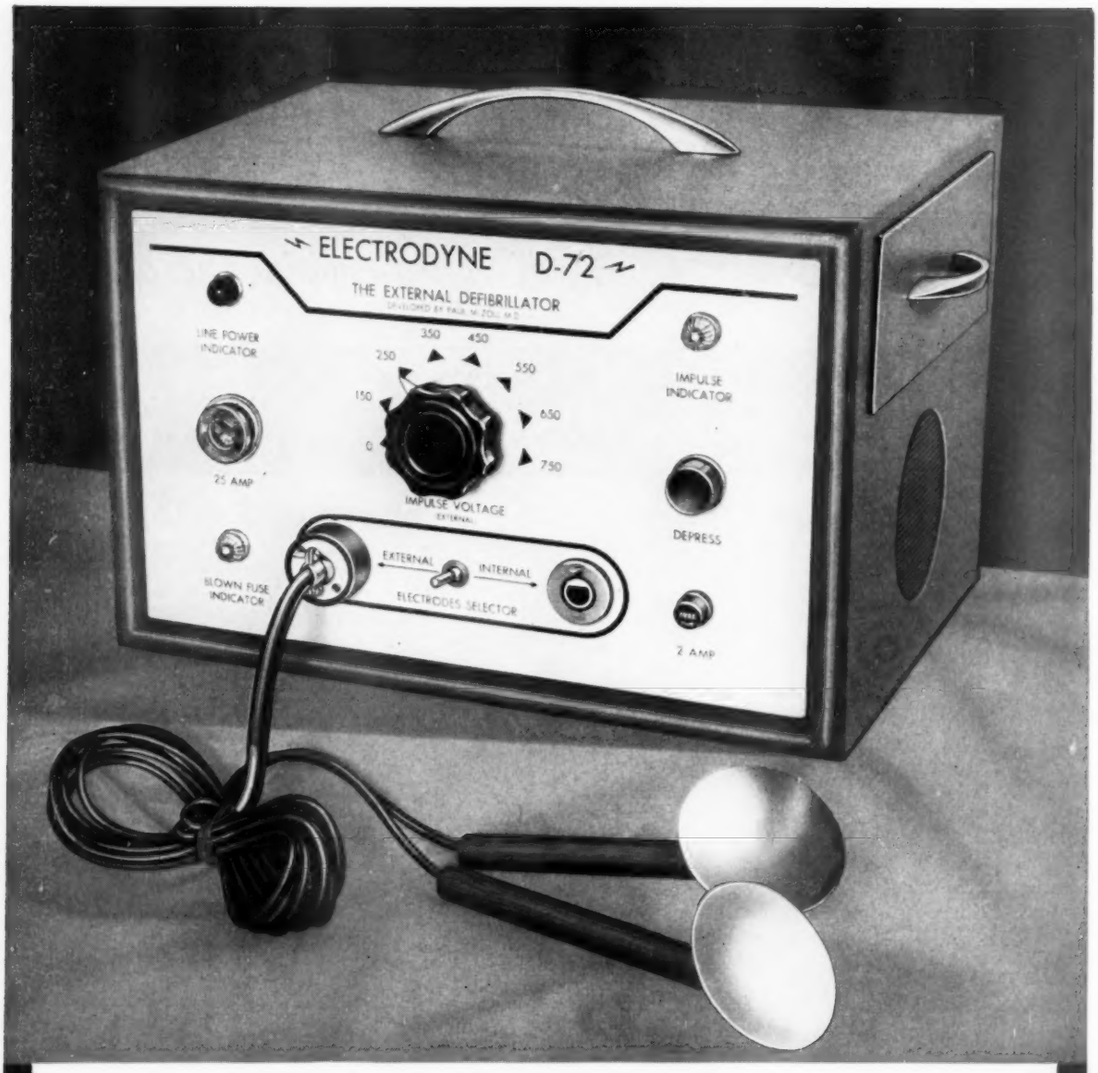

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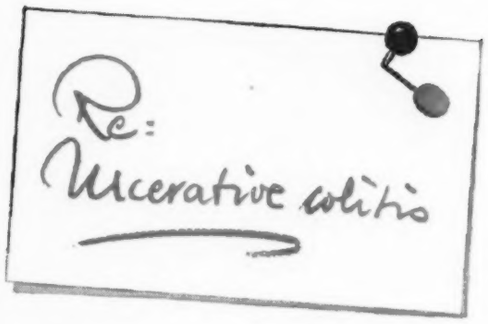

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1. BARGEN, J. A.: "Present Status of Hormonal and Drug Therapy of Ulcerative Colitis", South. M. J. *48*: 192 (Feb.) 1955.
2. BARGEN, J. A. and KENNEDY, R. L. J.: "Chronic Ulcerative Colitis in Children", Postgrad. Med. *17*: 127 (Feb.) 1955.
3. MORRISON, L. M.: "Response of Ulcerative Colitis to Therapy with Salicylazosulfapyridine", J. A. M. A. *151*: 366 (Jan. 31) 1953.

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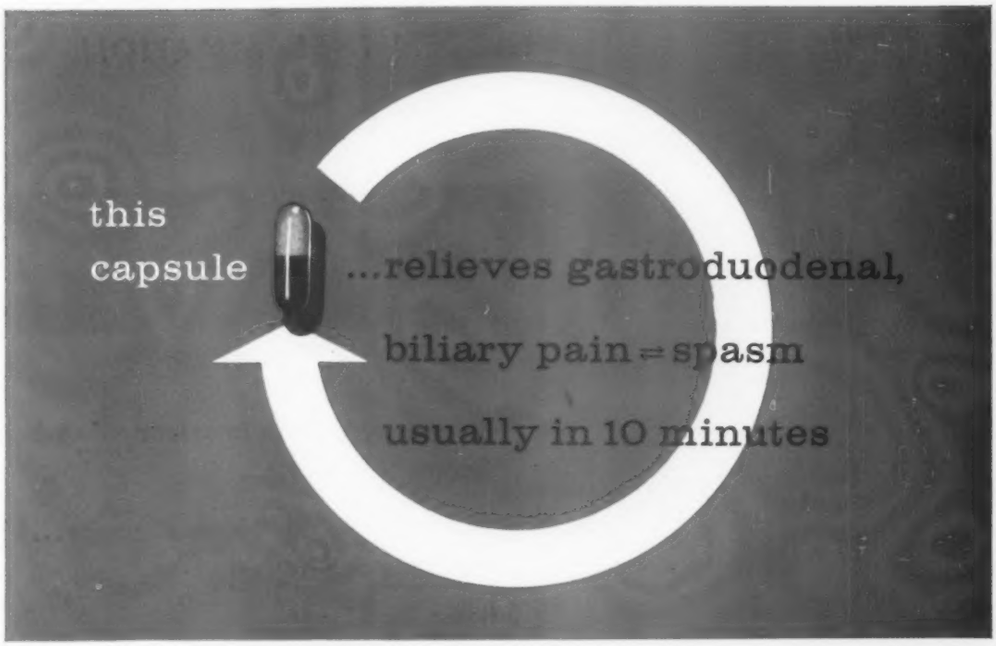

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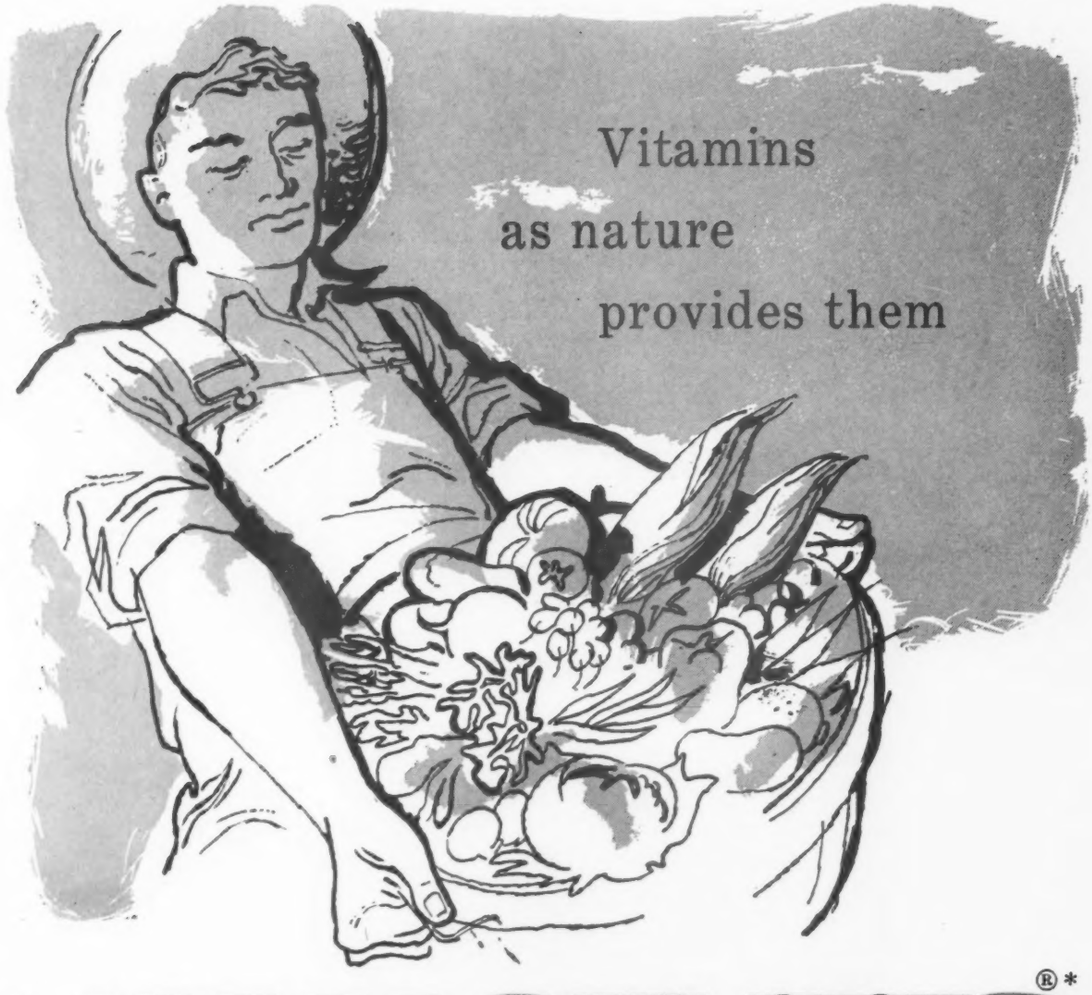

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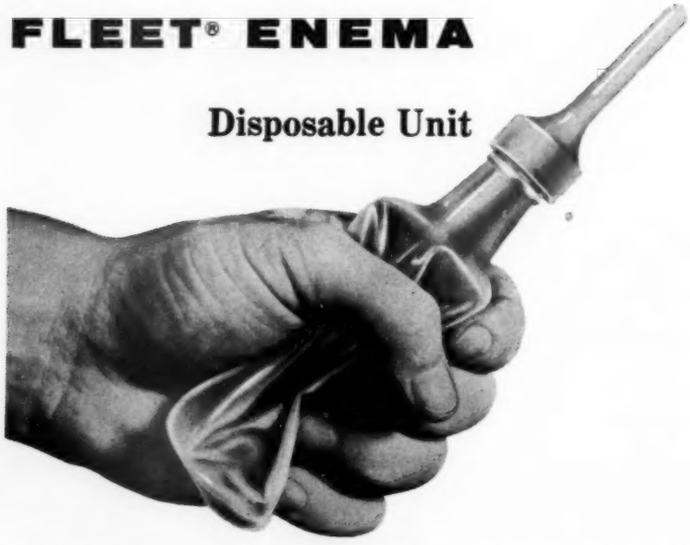

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(1) Swinton, N. W., Surg. Clin. of No. Am. 35:833, 1955
(2) Gross, J. M., Jl. Int. Coll. Surg. 23:24, 1955

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1. Groskloss, H. H. et al: Bonadoxin®: a unique control for nausea and vomiting of pregnancy. Clin. Med. 2:885 (Sept.) 1955. 2. Tartikoff, G.: The antiemetic function of Bonadoxin in the nausea and vomiting of pregnancy. Clin. Med. 3:223 (Mar.) 1956.

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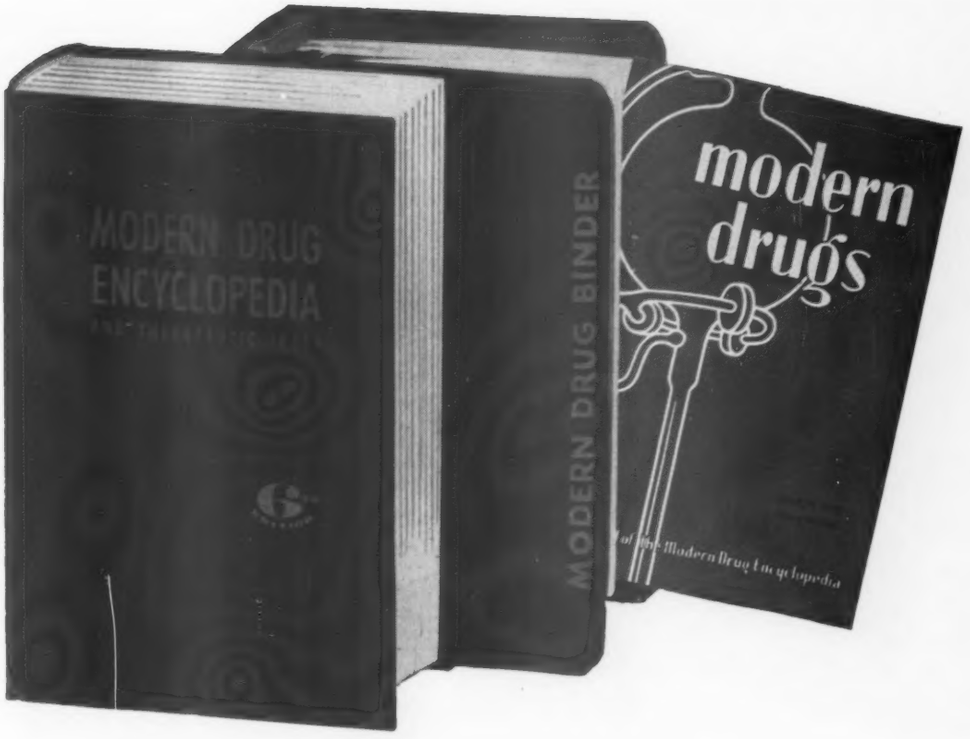

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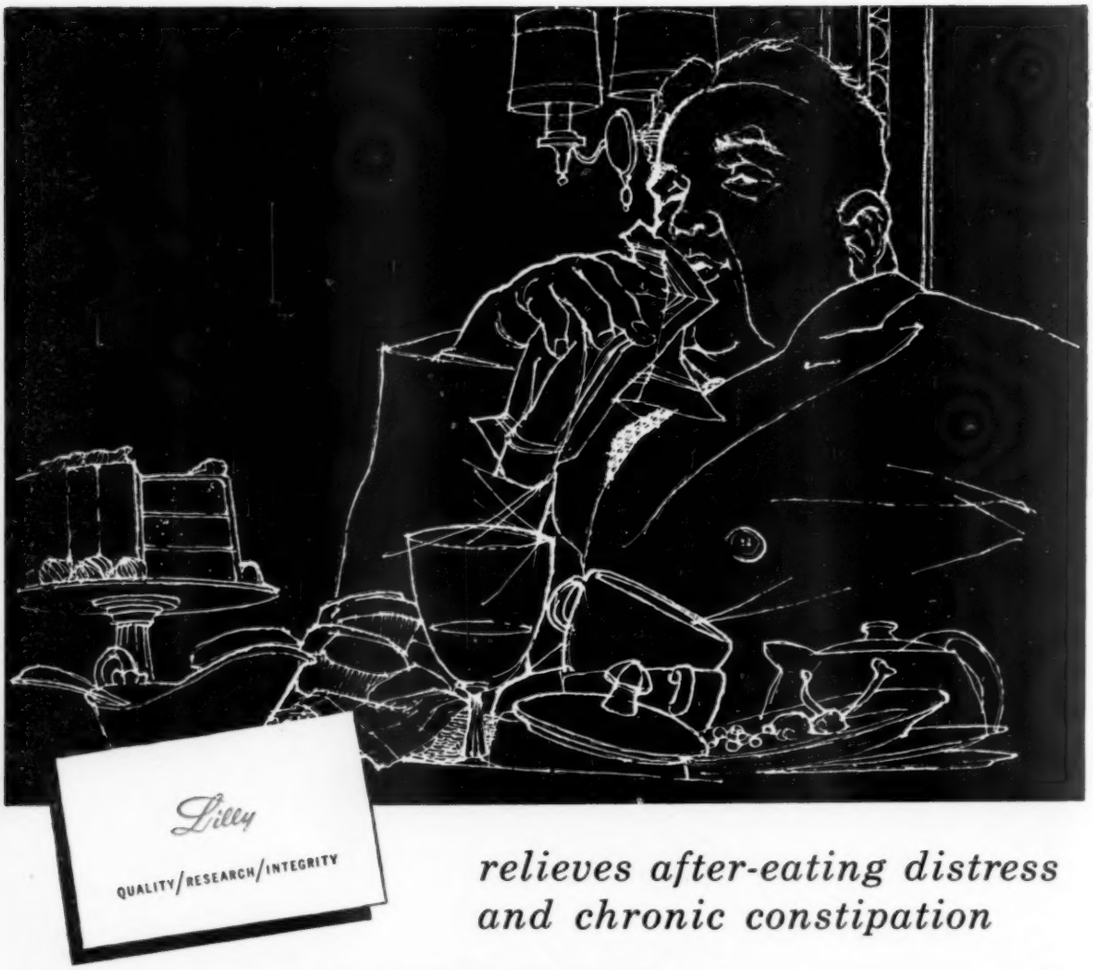

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The American Journal of Medicine

VOL. XXI SEPTEMBER, 1956 NO. 3

Editorial

Some Clinical Implications of the Spontaneous Diurnal Variation in Adrenal Cortical Secretory Activity

DIURNAL rhythm in the excretion of urinary 17-ketosteroids was first described in a group of young men by Pincus in 1943.[1] The same phenomenon was subsequently established for neutral reducing lipids.[2] This diurnal variation in the urinary excretion of adrenal cortical steroids has since been amply confirmed[3–5] and during the past five years it has been extended to include changes in plasma 17-hydroxycorticoids as well.[4–8]

The hourly urinary excretion of 17-hydroxycorticoids is usually maximal between 8 and 10 A.M. and minimal from 12 midnight to 2 A.M. Plasma levels of free 17-hydroxycorticoids closely parallel urinary excretion curves. The peak values for both at about 9 A.M. are two to three times greater than the minimal values close to 1 A.M.

Since approximately 80 per cent of the glucocorticoid secretion of the adrenal gland is cortisol and since 17-hydroxycorticoids are a measure of cortisol and its metabolites, they are the preferred metameter for following changes in adrenal cortical secretory activity. However, one cannot relate urinary total or plasma free 17-hydroxycorticoid levels to adrenal activity without considering the temporal and quantitative factors which characterize the disposal of cortisol. When hydrocortisone is given intravenously to a patient having undergone adrenalectomy who has normal liver and kidney function, 17-hydroxycorticoids are excreted in the urine at a rate which is characterized by an exponential function of time. The time for the excretion of one-half of the 17-hydroxycorticoid present in the organism at any one time is approximately four hours. This relatively slow rate of excretion leads to cumulative effects in urinary excretion and makes it impossible to relate a single urine or plasma value directly to concomitant adrenal cortical secretory activity. Using successive hourly urinary 17-hydroxy-

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corticoid excretions, however, it has been found possible to calculate approximations of hourly adrenal secretions of cortisol.[9] It was found that the adrenal secretes approximately 70 per cent of the daily secretion of cortisol under basal conditions from 12 midnight to 9 A.M. It would thus appear that the major portion of adrenal activity occurs in the early morning hours and during sleep. Indeed, a twofold to threefold change in secretory activity occurs quite regularly and abruptly and in apparent "anticipation" of the stress of daily existence. This diurnal cycle appears fixed for any given subject in any species, subject only to variations superimposed by changes in the external environment. These are mediated through the central nervous system and anterior pituitary adrenocorticotropin.[3] The cycle might conceivably be based upon cyclic discharges from the central nervous system or on rhythmic changes in the activity of the cells of the adrenal cortex and their enzyme systems. It is apparently abolished by hypophysectomy.[8] The fundamental mechanisms remain to be elucidated.

Cyclic variation in adrenal cortical activity is not confined to man. Dogs and Rhesus monkeys[10] have been studied by following changes in plasma 17-hydroxycorticoids, but only the monkey has shown striking diurnal variations in adrenal cortical activity. It has been studied exhaustively in mice by following changes in eosinophil levels known to be affected by adrenal cortical secretory activity.[11] This work has been extended to other white blood cells as well.[12] All of these cyclic changes are abolished by adrenalectomy. Just as in man, "anticipation" characterizes the cycle in mice. But in this species, one of nocturnal habits, the maximal adrenal cortical secretory activity occurs during the day rather than at night time.

What then determines the adrenal cortical cycle? Sleep habits *per se?* Probably not since in night workers the fundamental cycle did not change, in the studies of Migeon and Samuels[7] and in those of others.[8] Do visual impulses affect it? Presumably not since in studies on blind subjects no abnormalities in the cycle were noted.[7] Moderate food intake has little effect if any.[3,8] In contrast, emotion, stresses or painful stimuli upset the basic cycle[13] as the result of adding a corticotropin-induced rise in corticoid secretion. Unless a significant change in the diurnal cycle of adrenal cortical activity can be demonstrated under strictly basal conditions and in the absence of a stressful influence, a fundamental abnormality in the cycle cannot be invoked as the cause of disease. This trend of thought must be kept in mind when evaluating recent attempts to correlate changes in diurnal variation of adrenal cortical activity with the presence of collagen disease or with some related clinical manifestations.[14,15] Even if it may be difficult to demonstrate a change in the basic cycle in individuals and while no conclusive experimental proof for this has been presented as yet, one must keep an open mind in view of some of Halberg's fundamental findings.[16] He was able to correlate cyclic changes in the mitotic phases of liver tissue with the diurnal variation of adrenal cortical secretory activity in mice, observations pointing to possible future links between adrenal activity and connective tissue repair.

A number of practical clinical implications arise from the existence of a diurnal cycle of adrenal cortical secretory activity. When inhibition of adrenal cortical activity is desirable, as in the treatment of androgenic adrenal cortical hyperplasia, a dose of hydrocortisone or its derivative will prove nearly twice as effective when given at night as when administered in the morning. When adrenal cortical inhibition is to be prevented, as in the treatment of allergic or collagen diseases with small doses of corticoids, the manner of administration of

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[16] Halberg, F. Personal communication.

the dose will be critical in terms of the total corticoid supply achieved. Thus administration of 10 mg. of prednisolone in one dose at 8 A.M. will suppress spontaneous adrenal cortical secretory activity only during the hours of relative inactivity and thus become nearly additive to the twenty-four hour output from the adrenal glands; in contrast, the same dose given every six hours in divided doses will suppress endogenous adrenal secretory activity during the active period of adrenal cortical secretion as well, so that little will be gained by giving the drug in that manner. In low dose maintenance therapy two alternatives are thus presented: (1) suppression of endogenous adrenal cortical activity and of what might include harmful corticoids or (2) the addition of sorely needed corticoid at the time of relative lack without disturbing the remainder of the diurnal secretory cycle significantly. When going through the "weaning off" process after a high dose suppressive course of corticoids or corticotropin one would do well, once down to low dosage, to administer it all in one dose at 8 A.M. so as to allow the anterior pituitary corticotropin secretion to return gradually while supporting the patient with corticoid when receiving a minimal endogenous supply.

The concept of a cyclic variation in adrenal cortical activity is not usually taken into account when providing substitution therapy in Addison's disease. In fact, because of the stimulating effect that most patients experience when given corticoids, even in small amounts, the bulk is administered during the daytime and little if any is given in the evening. Thus the diurnal adrenal cortical cycle is reversed while eating habits remain the same. Could this lack of proper reproduction of diurnal variation account for the fact that persons with Addison's disease on a dose of corticoid barely sufficient to prevent appearance of pigmentation nonetheless show rounding of the face and obesity similar to the early changes of Cushing's syndrome? The lack of a proper mixture of hormones might be as important but the dynamic aspects must also be considered.

At the tenth anniversary of the demonstration of the spontaneous, diurnal variation in 17-hydroxycorticoid secretion by the adrenal cortex in man, with its anticipatory characteristics and apparent regulatory effect on the growth of somatic tissues, this emerges as an exciting physiologic phenomenon with potential pathologic ramifications and it carries a number of practical therapeutic consequences in the use of corticoids.

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PETER H. FORSHAM, M.D.
Department of Medicine, the Endocrine Clinic and the Metabolic Unit for Research in Arthritis and Allied Diseases
University of California School of Medicine
San Francisco, California

Clinical Studies

Insulin I-131 Metabolism in Man*

Plasma-Binding, Distribution and Degradation

GEORGE W. WELSH, III, M.D.,† ELAINE D. HENLEY, M.D., ROBERT H. WILLIAMS, M.D. *and* ROBERT W. COX, M.D.‡

Seattle, Washington

DIABETES mellitus is a disease implying a relative or absolute deficiency of insulin. Many persons with diabetes require daily insulin in excess of that necessary in depancreatized man. Certain middle-aged obese diabetic persons (maturity-onset type) have 70 per cent of the normal level of measurable circulating insulin[1] and also near-normal levels of pancreatic insulin.[2] Morphologic changes in the pancreas are often absent or minimal.[3,4] On the other hand persons with juvenile diabetes (growth-onset type) more frequently have markedly decreased circulating and pancreatic insulin levels, reflecting an absolute deficiency of hormone.[1,2]

The diabetic state provoked in animals by excessive feeding of carbohydrates[5] or administration of growth hormones[6,7,8] or adrenocorticotrophic and adrenocortical hormones[9,10] may be preceded by initial overproduction of insulin, followed by degranulation and degeneration of the beta cells. Since the middle-aged, obese person with diabetes is relatively insulin-insensitive compared to most persons with juvenile diabetes, it has been conjectured that contra-insulin factors may play a part in both the etiology and course of this type of diabetes. Among the factors to be considered are (1) endocrine antagonists—the pituitary and adrenal hormones, (2) increased destruction or rejection of insulin at the cellular level, (3) immunologic mechanisms—the development of antibodies to exogenous or even endogenous insulin, and (4) unknown extracellular factors tending to impede the distribution of insulin to the tissues.

In the past, efforts to study insulin metabolism have been beset with many difficulties, chiefly because of the minute concentrations of the hormone and the difficulty of accurately assaying the effect of insulin on carbohydrate metabolism. The development of isotope technics has made it possible to trace small quantities of labeled insulin in the living organism and to study its distribution and eventual fate.[11–13] This study was undertaken to determine differences, if any, in the metabolism and distribution of insulin I-131 in persons with and without diabetes, and to investigate the cause and significance of such differences.

It was found that intravenously injected labeled insulin was retained in the plasma of many insulin-treated persons with diabetes longer than in those without diabetes. *In vitro* plasma binding was demonstrated to be correlated grossly with the *in vivo* abnormalities. In those instances tested, plasma capable of binding insulin was shown to have a definite protective influence against insulin hypoglycemia in mice. A plasma-binding factor is thus proposed as affecting insulin metabolism in many insulin-treated persons with diabetes.

MATERIALS AND MEASUREMENTS

Patients and house staff from the King County Hospital and the Veterans Administration Hospital were used in this study. Unless otherwise noted, all subjects fasted from midnight prior to the study and

* From the Department of Medicine, University of Washington School of Medicine, Seattle, Washington. Supported in part by grants-in-aid from the United States Public Health Service, the Atomic Energy Commission and The Upjohn Company.

† Present address: Burlington, Vermont.

‡ Present address: Walla Walla, Washington.

insulin was withheld from diabetic subjects for twenty-four hours.

Tissues from adult non-fasted male and female Sprague-Dawley rats weighing between 200 and 300 gm. were used for *in vitro* studies. The animals were sacrificed by cerebral concussion, and the tissues were removed immediately and placed in 0.067 M phosphate buffer (pH 7.5) at 5°c. Whole diaphragms, trimmed of non-muscular tissue, were quartered and again trimmed to equalize weights. One-quarter of each diaphragm was used in each of four incubation media to allow comparison with a control within each experiment. Liver slices of approximately equal size, thickness and weight were prepared. Liver homogenate was freshly prepared by grinding a weighed aliquot of liver in a Misco homogenizer with phosphate buffer. Male white Swiss mice weighing 15 to 25 gm. fasted for five hours, were used for bio-assay of insulin hypoglycemic activity. Blood samples (0.1 ml.) were obtained from the tail vein by aspiration in a heparinized Normax capillary pipette.

Insulin. Highly purified crystalline zinc insulin (beef),* assayed at 26 to 27 units per mg., was radioiodinated by the Abbott Laboratories at Oak Ridge, Tennessee. The degree of iodination and the properties of insulin I-131 have been previously described in detail in publications originating from this laboratory.[11,14] The specific activity ranged between 332 and 3,798 microcuries per mg. The radioactivity of the insulin I-131 solution was 85 to 95 per cent precipitable with trichloroacetic acid (TCA) in the presence of a 2 per cent solution of dried human plasma. After dialysis for 12 to 16 hours against 0.067 M phosphate buffer at 5°c., 97 to 99 per cent of the total radioactivity was precipitable with TCA.

Amorphous insulin, assayed at 21 units per mg. was used in the *in vitro* and bio-assay experiments.

Radioactivity. Since intact insulin I-131 is precipitable by trichloroacetic acid (TCA), it was assumed that all radioactivity so precipitated from plasma was bound to insulin. It has been shown by Tomizawa et al.[14] that increase of TCA-soluble radioactivity resulting from incubation of a mixture of amorphous and labeled hormone with liver homogenate is associated with an increase in the non-protein nitrogen and a proportional decrease in biologic activity, implying proteolysis of the insulin molecule and concurrent biologic inactivation. Accordingly, it was assumed that all TCA-soluble (non-protein–bound) radioactivity represented degradation products of insulin I-131 and that all TCA-precipitable (protein-bound) radioactivity represented intact insulin I-131. This assumption was made for convenience in interpreting the results.

The method of precipitating samples of TCA and preparing them for measurement of radioactivity has been described in detail previously by Elgee et al.[11]

* Supplied by Dr. O. K. Behrens of the Eli Lilly Company.

Epithyroid radioactivity in human subjects was estimated with a scintillation counter and compared to appropriate standards.

Plasma Volume. In order to estimate the dilution of the dose of insulin I-131, the plasma volume of human subjects was calculated from the height and weight (using the DuBois Tables for derivation of body surface area), the venous hematocrit and the factor 3.3 L. blood volume per square meter of body surface.[15] While this method is not as accurate as more direct measurements of plasma volume, it served to eliminate some of the variables between subjects and allowed closer correlation of results in the control series. The protein-bound and non-protein–bound radioactivity in the plasma and urine could then be expressed as a percentage of the respective radioactivity in the administered dose.

Blood Sugar. The glucose content of the tail vein blood of mice was determined by the method of Nelson and Somogyi.[16,17]

IN VIVO STUDIES

A total of 118 *in vivo* experiments were conducted in 106 subjects, forty-three without and sixty-three with diabetes. (Table 1.)

Fasted subjects were given non-dialyzed insulin I-131 in physiologic saline solution intravenously over a two minute period. The administered radioactivity varied between 50 and 200 microcuries, with insulin dosage ranging from 0.5 to 5.0 units. At the time of injection each subject was given 10 gm. of glucose *per os* per unit of insulin administered. At predetermined times venous blood samples were withdrawn in heparinized syringes and centrifuged. The plasma was removed and processed with TCA before assaying for radioactivity. It has been shown previously that the binding of insulin I-131 by erythrocytes is negligible.[11,18] In many subjects the cumulative sixty-minute urinary excretion of radioactivity was measured, and in some subjects epithyroid radioactivity was measured one hour after injection.

Non-diabetic Control Subjects. The rate of disappearance of intact insulin I-131 (TCA-precipitable radioactivity) from the plasma of thirty-nine non-diabetic subjects is shown by the solid curve in Figure 1. At three minutes after beginning the intravenous injection the maximum concentration of intact labeled insulin in any subject's plasma was 72 per cent. Variations in the plasma concentrations were large in the initial rapid distribution phase for fifteen minutes following injection but became progressively smaller during the later slower distribution

TABLE 1

CHARACTERISTICS OF HUMAN SUBJECTS

Subject No.*	Sex	Dose Insulin I-131 in Plasma (% at 60 min.)	Age (yr.)	Duration Diabetes (yr.)	Insulin Requirements (units)	Diabetic Complications			Other Observations
						Vascular	Neuropathy	Nephropathy	
Non-Diabetic Control Group									
1	F	4.5	44						Chronic cholecystitis
2	M	5.0	43						Anxiety neurosis
3	F	5.3	35						Uterine fibroids, hysterectomy
4	M	5.9	39						Rheumatic heart disease, inactive
5	M	6.0	27						Carcinoid tumor of stomach
6	M	6.3	27						Healthy
7	M	6.5	25						Healthy
8	F	6.6	49						Benign hypertension
9	F	6.6	21						Pregnancy; epilepsy
10	M	6.9	29						Healthy
11	M	6.9	59						Arteriosclerotic heart disease; syphilis, latent
12	M	7.0	50						Cirrhosis of the liver, mild
13	M	7.1	28						Healthy
14	M	7.5	31						Residual hemiplegia
15	M	7.5	30						Healthy
16	F	7.5	34						Cirrhosis of the liver, severe
17	F	7.5	61						Metastatic carcinoma breast
18	M	7.5	34						Multiple sclerosis, inactive
19	M	8.0	33						Healthy
20	M	8.0	26						Healthy
21	M	8.5	23						Active rheumatic carditis; ACTH therapy
22	M	8.5	64						Insulinoma (?)
23	F	8.6	14						Ovarian cyst
24	F	8.7	25						Carcinoma *in situ;* hysterectomy
25	M	8.7	51						Chronic pyelonephritis
26	F	9.0	45						Uterine fibroids; hysterectomy
27	F	9.0	46						Cirrhosis of the liver, severe
28	M	9.4	30						Sarcoid
29	M	9.5	56						Pancreatitis; alcoholism
30	F	10.0	22						Incomplete abortion
31	M	10.0	36						Chronic alcoholism
32	F	10.0	59						Thyrotoxicosis
33	M	10.2	45						Reactive depression; bronchitis
34	F	10.5	21						Induced abortion
35	M	11.0	23						Rheumatic heart disease, inactive
36	F	11.0	85						Carcinoma of the colon; during surgery under anesthesia
37	M	12.0	70						Pneumonia; carcinoma of the prostate; uremia
38	M	13.0	48						Rheumatoid arthritis, cortisone therapy
39	M	13.0	41						Rheumatic heart disease, active (?)
Other Non-Diabetic Subjects									
40	M	18.0	90						Chronic pyelonephritis; uremia
41	M	18.0	51						Acromegaly, arrested
42	M	26.0	37						Schizophrenia†
43	M	33.0	25						Schizophrenia†

*See footnote on page 328.

TABLE 1 (*Continued*)

Subject No.*	Sex	Dose Insulin I-131 in Plasma (% at 60 min.)	Age (yr.)	Duration Diabetes (yr.)	Insulin Requirements (units)	Diabetic Complications			Other Observations
						Vascular	Neuropathy	Nephropathy	
Diabetic Control Group‡									
44	M	6.0	25	17	30	++	+	0	None
45	M	6.2	39	2	Untreated	0	0	0	Obesity
46U	M	6.2	37	weeks (?)	Untreated	0	0	0	Obesity
47U	M	7.5	37	weeks (?)	Untreated	0	0	0	Obesity
48	F	7.5	60	4	20	+	+	+	None
49	F	7.5	36	2 mo.	12	0	0	0	None
50	F	7.5	66	3 mo.	35	+++	0	0	Hypertensive cardiovascular disease; cerebrovascular accident
51N	M	9.0	66	23	35	++++	++	+	Leg amputation
52	F	9.4	57	8	15	++++	+	+	Leg amputation
53N	M	10.0	66	23	35	++++	++	+	Leg amputation
54	F	10.0	84	3	25	++	+	+	Arteriosclerotic heart disease
55	F	10.5	76	31	70	+++	++	+	Arteriosclerotic heart disease
56A	M	10.5	29	11	60	+	+	+	Upper respiratory infection; epilepsy
57	M	12.0	45	4	40	+++	0	++	Hemochromatosis
58	F	12.7	36	weeks (?)	Untreated	0	0	0	Obesity
59	M	13.0	8	10	35	+++	+	+	None
60F	F	13.1	66	1½	Untreated	0	0	0	Carcinoma of the breast
61	M	13.2	67	3 weeks	Untreated	0	0	0	None
62D	M	14.0	23	9	60	+	++	+	Leg ulcers
63	F	14.0	64	1½	24	+	+	+	Nodular goiter, non-toxic
64	M	14.0	73	5 (?)	Untreated	++	++	++	None
65	M	14.0	77	7	40	+	+	+	Pneumonia
66L	M	14.5	36	18	80	++	+	+	None
67	M	15.0	33	9	60	+	++	+	Leg ulcer
68	M	15.0	50	5 mo.	35	++	+	+	Hemochromatosis
69	M	15.4	63	2	10	+++	+	++++	Chronic pyelonephritis; uremia
70L	M	15.5	36	18	60	++	+	+	None
71	F	16.0	33	2½	24	+	+	+	None
72	F	17.0	47	5	0	+	+	+	Hypertensive cardiovascular disease
73	M	17.0	51	6	40	+	+	+	None
74	F	18.0	71	20	85	+++	+	+	None
75	F	19.7	65	22	30	+	+	+	None
76	M	20.0	64	1	30	+	+	+	None
77T	M	21.0	42	6	60	+	+	+	Lung abscess, healing
78F	F	22.0	66	1½	20	+	+	+	Carcinoma of the breast
79	F	23.0	46	3	15	+	+	+	Acromegaly, untreated
80	M	24.0	66	4	15	+++	+	++++	Hypertensive cardiovascular disease
81	M	24.0	55	2	40	+	+	+	Mental defective
82	F	24.0	19	5	56	+	+	+	Upper respiratory infection
83X	M	25.0	54	8	30	+	+	+	None
84B	M	25.0	34	10	38	+++	+	0	Mental defective
85A	M	27.0	30	12	60	+	+	+	Epilepsy
86	M	27.0	44	15	40	+	+ Acidosis	+	Alcoholism
87	M	29.0	73	14	10	+++	++	+	Latent syphilis
88T	M	30.0	42	6	42	+	+	+	Lung abscess, healing
89	F	30.0	58	10	50	++	+	+	Arteriosclerotic and hypertensive cardiovascular disease

*See footnote on page 328.

TABLE 1 (*Continued*)

Subject No.*	Sex	Dose Insulin I-131 in Plasma (% at 60 min.)	Age (yr.)	Duration Diabetes (yr.)	Insulin Requirements (units)	Diabetic Complications			Other Observations
						Vascular	Neuropathy	Nephropathy	
90	F	30.0	38	4	48	+	+	+	None
91	F	30.0	55	4	40	+	+	+	None
92	M	30.0	17	4	80	+	+	+	None
93	F	30.0	28	10	50	+	+	+	None
94B	M	33.0	34	10	65	+++	+	+	Mental defective
95	F	35.0	64	27	60	+++	+	+	Leg amputation
96	M	40.0	37	2	110	+	+	+	Acromegaly, arrested
97	M	40.0	17	11	80	+	+	+	Cellulitis
98	F	40.0	46	8	8	+	+	+	Arteriosclerotic and hypertensive cardiovascular disease
99	F	43.0	69	10	50	+	+ Ketosis	+	Obesity
100	M	46.0	42	24	64	+++	+	+	Arteriosclerotic and hypertensive cardiovascular disease
101X	M	47.0	54	8	50	+	+	+	None
102	M	49.0	21	13	40	++++	+	++++	Uremia
103V	F	50.0	54	3	0	++	+	++++	Chronic cholecystitis; hypertensive cardiovascular disease
104	M	52.0	34	11	50	+	++++	+	None
105	M	54.0	26	8 mo.	40	+	+	+	Postpancreatectomy; insulinoma
106	M	59.0	50	2	18	+	+	+	Calcified head of pancreas
107S	M	60.0	70	6	40	+++	++	+	Leg amputation
108S	M	62.0	70	6	40	+++	++	+	Leg amputation
109	F	62.0	43	17	34	++	+	++	Hypertensive cardiovascular disease
110	M	63.0	71	20	34	++	+	+	None
111	M	65.0	64	11	50	++	+	+	None
112E	M	72.0	33	12	60	+++	++	+++	Pyelonephritis, chronic
113	F	75.0	30	7	34	++	+	+	None
114E	M	79.0	33	12	60	+++	++	+++	Pyelonephritis, chronic
115E	M	81.0	33	12	60	+++	++	+++	Pyelonephritis, chronic
116	M	85.0	83	7	40	+	+	+	Cystitis; arteriosclerotic heart disease
117V	F	90.0	54	3	0	++	+	++++	Pancreatitis; cholecystitis; hypertensive cardiovascular disease
118	M	90.0	61	23	50	++++	+	++	Leg amputation

* Letters accompanying subject numbers indicate duplicate experiment on same subject.

† Insulin shock therapy four months.

‡ Complications are indicated in the following manner: + = minimal; ++ = mild; +++ = moderate; ++++ = severe.

phase. Therefore at sixty minutes between 4.5 and 13 per cent of the dose remained in the plasma. At 180 minutes 3.6 per cent still remained, while at twenty-four hours only 0.5 per cent of the dose could be measured in the plasma of one subject. Of the control series, seven subjects were in excellent health, nineteen were convalescing from surgery or minor illnesses and thirteen had severe illnesses. It is noteworthy that in all the ill subjects labeled insulin disappeared as rapidly from the plasma as in the seven entirely healthy subjects. Of particular interest are the results in patients with severe disease of the liver and kidney. The studies of Weisberg et al.[19] showed that less hypoglycemia was present in dogs following insulin injection into the splenic vein than when administered into the femoral vein, indicating

that liver inactivates insulin. Studies of rats by Elgee et al.[11] have shown that the liver and kidneys are major sites of concentration and of subsequent degradation of insulin; hence it was postulated that extensive pathologic changes in either organ might be reflected in increased retention in the plasma and decreased degradation of insulin I-131. However, in two patients (Nos. 16 and 46) who had severe cirrhosis of the liver and in two others (Nos. 25 and 37) who had severe disease of the kidney, labeled insulin was not retained in the plasma to any greater degree than in the others in this series. On the other hand, in a third subject (No. 40) who had severe disease of the kidney 18 per cent of the labeled insulin dose was retained in the plasma at sixty minutes, which is significantly higher than that of the non-diabetic control group as a whole and may be due to failure of the kidneys to concentrate and degrade labeled insulin. The only other non-diabetic subject showing a significantly elevated plasma level of labeled insulin was an acromegalic (No. 41) in whom 18 per cent was retained at sixty minutes. This subject had never been treated with insulin and had never showed signs, symptoms or laboratory evidence of diabetes mellitus. These two latter subjects are not included in the control group. Other metabolic abnormalities, such as thyrotoxicosis, and ACTH or steroid therapy did not retard the rapid disappearance of labeled insulin from the plasma.

Degradation Products of Insulin I-131 (TCA Supernatant Radioactivity). Although the administered labeled insulin solution initially contained between 5 and 10 per cent non-precipitable radioactivity, little of this activity was recoverable from the plasma within 3 to 4 minutes after beginning the injection and presumably was rapidly diffused out of the blood stream. However, as shown in Figure 1, this fraction then increased rapidly to an average of 11.4 per cent at sixty minutes and then slowly decreased. This suggests that the degradation products of insulin I-131 enter the blood stream faster than they are cleared.

Urinary radioactivity excretion was measured in sixteen control subjects, and averaged 10 per cent of the administered dose at sixty minutes. The twenty-four hour excretion, measured in eight control subjects, averaged 73 per cent. Ninety-five per cent of the urinary radioactivity was non-precipitable with TCA and therefore constituted degradation products of labeled insulin, which agreed with the findings of Mirsky et al.,[21] Cutting[20] and Elgee et al.[11] that a negligible amount of insulin is excreted in the urine.

Epithyroid radioactivity concentration, measured sixty minutes after injection of labeled

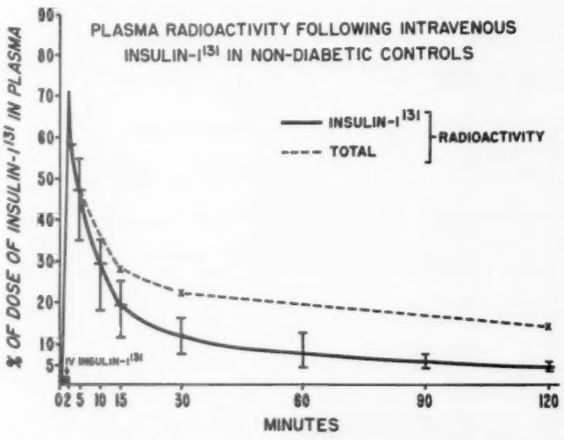

FIG. 1. Insulin I-131 disappeared rapidly from the plasma of thirty-nine non-diabetic subjects after intravenous administration. The brackets indicate the range of values at each time interval after injection. At 60 minutes an average of 8.3 per cent of the dose of labeled insulin remained in the plasma. Degradation products of insulin I-131 constitute the difference in values between the total radioactivity and the insulin I-131 (protein-bound) radioactivity curves.

insulin, in nine control subjects averaged 9 per cent of the administered dose.

These findings suggest that labeled insulin not only disappears rapidly from the plasma of non-diabetic subjects but that it is also degraded rapidly by the tissues and that the degradation products are cleared by the kidney. The increase in thyroid radioactivity suggests that I-131 is eventually released after degradation of labeled insulin and is concentrated in the gland.

Diabetic Subjects Not Receiving Insulin Therapy. Six subjects (Nos. 45, 46U, 58, 60F, 61, 64) with diabetes had never received insulin therapy. At sixty minutes the subjects in this group had an average of 11 per cent of the dose of labeled insulin remaining in the plasma and an additional 10 per cent of the radioactivity in the degradation products fraction. These values are within the range of results obtained in the control series.

Insulin-Treated Diabetic Subjects. Fifty-seven patients with diabetes who had been treated with insulin for at least six weeks were studied.

In twelve of these a greater percentage of labeled insulin was not retained in the plasma than was retained in the subjects in the control series. On the other hand, forty-five (79 per cent) of the group showed significant retardation in the rate of disappearance of labeled insulin

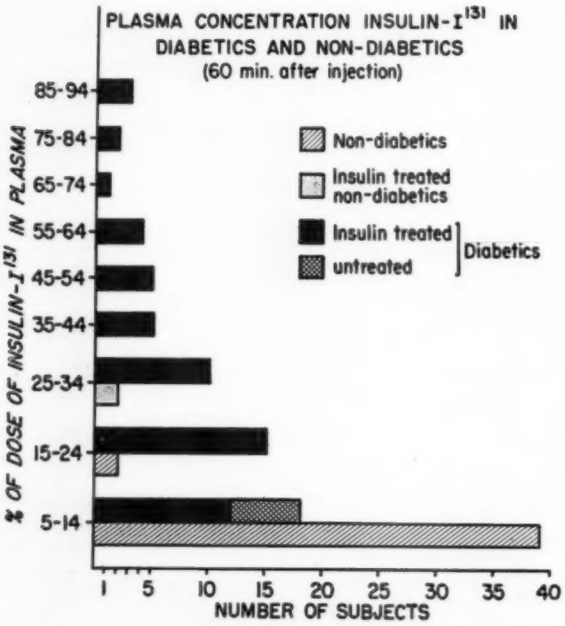

FIG. 2. Illustrating the number of non-diabetic and diabetic patients who retained different percentages of the dose of insulin I-131 in their plasma 60 minutes after injection. Whereas thirty-nine controls and eighteen diabetics (including six untreated) retained between 5 to 14 per cent, forty-five insulin-treated diabetics and two schizophrenics retained 15 to 90 per cent of the dose.

from the plasma. At sixty minutes between 15 and 90 per cent of the dose of labeled insulin remained circulating in the plasma. Figure 2 illustrates the number of subjects who fall into each tenth percentile group. Only four non-diabetic subjects are in the diabetic group. Two of these have been discussed with the control series and the other two were insulin shock-treated schizophrenic subjects who are to be considered subsequently. Individual curves of plasma concentration of labeled insulin in the persons with diabetes (not illustrated) show that the retarded rate of distribution occurs mainly in the early period following injection so the curves are much flatter.

Figure 3 illustrates that proportionally less degradation products of labeled insulin were present in the urine and plasma of subjects exhibiting increased retention of intact labeled hormone in the plasma. The twenty-four hour urinary excretion of radioactivity was measured in three diabetic persons and averaged 55 per cent, compared to 73 per cent in eight control subjects. Epithyroid radioactivity was measured in six diabetic persons and averaged

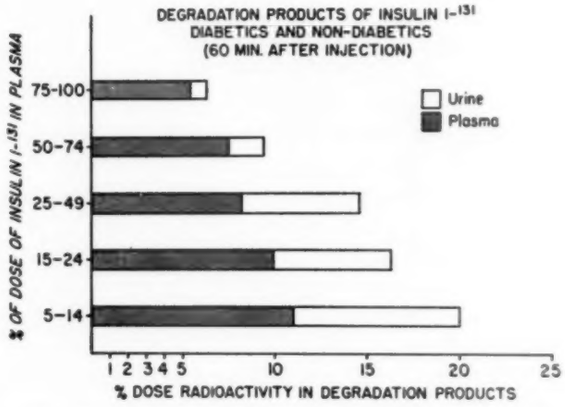

FIG. 3. The relationship between the amount of intact insulin I-131 retained in the plasma (ordinate) and the total measurable degradation products in the plasma and urine 60 minutes after injection. There are proportionally less degradation products in both the plasma and urine when there is greater retention of intact labeled hormone in the plasma, indicating protection from degradation.

5.6 per cent, compared to 9 per cent in nine control subjects.

Correlation of Results with Clinical Characteristics of Subjects. Table 1 lists the pertinent characteristics of the diabetic patients. These data are arranged in order of increasing percentage of labeled insulin retained in the plasma at sixty minutes, in order to assess any correlation between clinical characteristics and the observed experimental results. It has already been noted that six patients with diabetes who had not received insulin therapy showed no increased retention of labeled insulin in the plasma. However, one subject in that group (No. 60F), while retaining 13 per cent of the dose prior to insulin therapy, was retested six weeks after insulin therapy was started and plasma retention was found to have increased 22 per cent (No. 78F).

In the forty-five insulin-treated diabetic subjects who retained more than 15 per cent of the dose of labeled insulin in the plasma at sixty minutes marked variations were noted in age, duration of diabetes, insulin requirements, blood sugar content and degree of vascular, renal and neural complications of diabetes.

None of these variable characteristics could be correlated with the abnormalities disclosed by the experimental procedure.

Some of the insulin-treated diabetic subjects warrant individual comment. In subject No. 105, in whom diabetes secondary to subtotal pancreatectomy had developed and who had been treated with 40 units of insulin for eight months, 54 per cent of the dose of labeled insulin was retained in the plasma at sixty minutes. In subject No. 117V, who required no therapeutic insulin at the time of testing, 90 per cent of the dose was retained in the plasma at sixty minutes. This patient, however, was subject to recurrent attacks of pancreatitis during which hyperglycemia and glycosuria developed to such an extent that intermittent insulin therapy was required. In neither of two subjects with diabetes secondary to hemochromatosis (Nos. 68 and 57) were increased percentages of labeled hormone retained in the plasma. Five labile diabetic patients (Nos. 56A, 73, 94B, 104, 115E) were given labeled insulin to determine whether or not insulin sensitivity was reflected in rapid disappearance of the labeled hormone from the plasma. Plasma retention varied as markedly as in the entire series of the diabetic subjects chosen at random. However, in one labile diabetic person 10 per cent of a dose was retained in the plasma while convalescing after diabetic acidosis (No. 56A), but ten months later 27 per cent (No. 85A) was retained. In the interim the subject's insulin requirements had not changed and the clinical condition had been good, but satisfactory control of alternating hyperglycemia and hypoglycemia had become more difficult to achieve.

Insulin Shock-Treated Schizophrenic Persons. In order to investigate the effect of insulin administration *per se* on the plasma retention of labeled insulin, two schizophrenic patients were studied. Each had received courses of insulin-shock therapy for four months prior to testing with labeled insulin. Subject No. 42 retained 26 per cent and subject No. 43 retained 33 per cent of the dose in the plasma at sixty minutes. Neither of these patients exhibited signs or symptoms of diabetes mellitus prior to or after insulin-shock therapy.

Variations of Experimental Conditions. Varying the dose of insulin in four subjects who were tested on two occasions did not alter the results, the curves of plasma insulin I-131 concentration being virtually superimposable.

To investigate the possibility of enhancing plasma retention of labeled insulin, therapeutic insulin was withheld from one subject for ninety-six hours (No. 101X). This resulted in 47 per cent plasma retention compared with 25 per cent two months previously when insulin was withheld for only twenty-four hours (No. 83X). In the interval between tests this patient's condition had become more difficult to control and the insulin requirement had increased from 30 to 50 units. In another subject 79 per cent of the dose was retained after insulin was withheld for forty-eight hours (No. 114E) and 72 per cent and 81 per cent on two other occasions (Nos. 112E and 115E) when insulin was withheld for only twenty-four hours. Clinically, this patient's insulin requirements and course had been relatively stable over the eight months during which the three tests were performed.

Conversely, the consideration that plasma retention of labeled insulin might be decreased by insulin loading of diabetic patients prior to testing was investigated. In three non-fasting subjects therapeutic insulin was not withheld, and fifteen minutes prior to administration of labeled insulin each subject was given 0.1 unit of regular insulin per kilogram of body weight intravenously. One subject, in whom 60 per cent was retained under standard experimental conditions two days previously (No. 107S), retained 62 per cent with insulin loading (No. 108S). Another subject in whom 23 per cent was retained under standard conditions six weeks earlier (No. 77T) plasma retention increased to 30 per cent with an insulin load (No. 88T). In a third subject in whom 33 per cent was retained six weeks earlier (No. 94B) retention decreased to 25 per cent with loading (No. 84B).

Consideration of these data demonstrates a single common factor, namely, that in the majority of human subjects who have received insulin therapy, insulin I-131 is retained in the plasma longer and to a greater degree than in subjects who have not received therapeutic insulin. Alterations in procedure, in terms of loading or withdrawal of therapeutic insulin, or varying the dosage of labeled insulin did not change the results in any consistent manner. Differences in degree of plasma retention were noted over time intervals of weeks or months, which may suggest a changing response to insulin therapy.

Interpretation of the results suggested that in

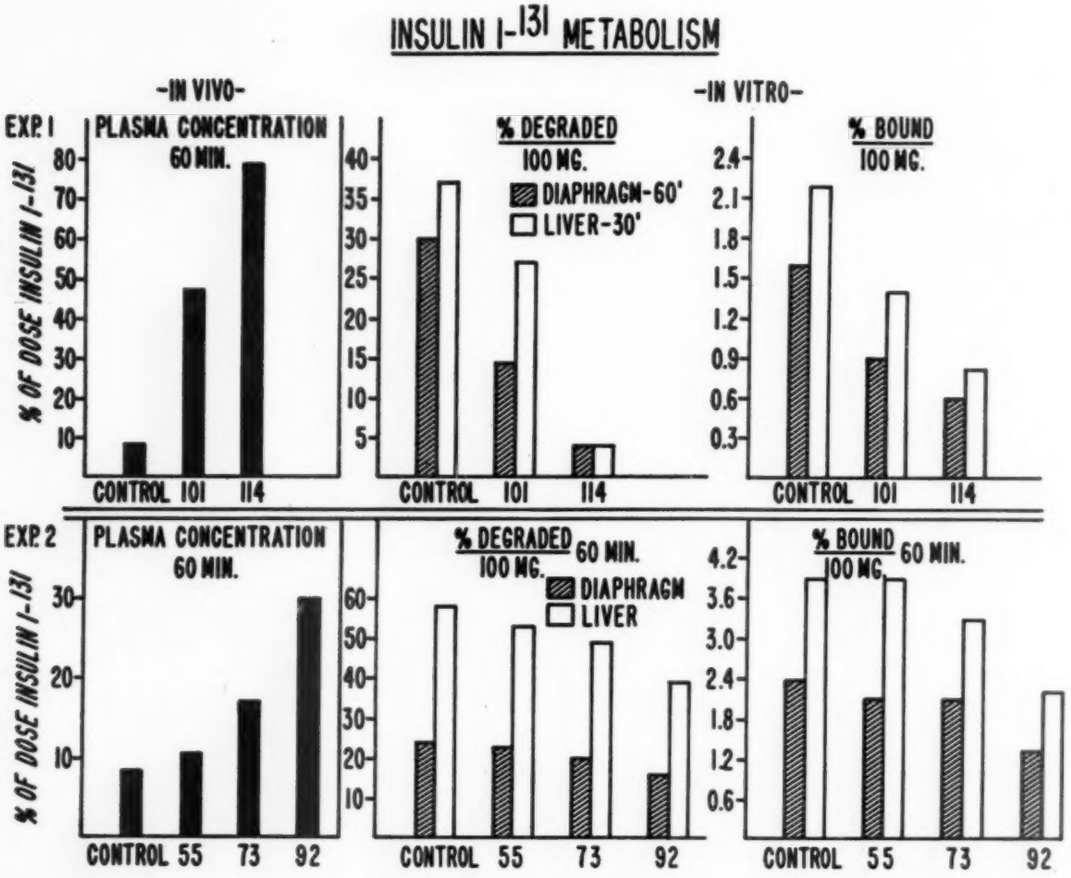

FIG. 4. The correlation between the *in vivo* plasma retention of insulin I-131 60 minutes after injection and the tissue-binding and degradation of labeled insulin *in vitro*. Two experiments with separate controls are shown. The controls and diabetic subject numbers are noted on each abscissa. Plasma from diabetic subjects in each experiment proportionally reduces the amount of labeled insulin bound to and degraded by rat liver and diaphragm, compared to each control. The greater the plasma retention *in vivo*, the less is the amount of insulin I-131 bound and degraded by tissues *in vitro*. Percentages of insulin I-131 doses *in vitro* are expressed per 100 mg. of tissue and time intervals of incubation are indicated.

insulin-treated patients either insulin is bound in the plasma or rejected at the cellular level. Either instance might well decrease effective biological activity.

To separate these alternatives an *in vitro* experimental system was devised. It has been shown by Stadie[22] that insulin will bind firmly to rat diaphragm and Mirsky[23,24] has demonstrated that labeled insulin is degraded by liver slices and homogenate and by muscle. If there were factors in plasma that would bind insulin, the amount of degradation of labeled insulin might be decreased and less labeled insulin might be available for binding to tissues.

IN VITRO STUDIES

In these experiments 1 ml. of phosphate buffer solution (pH 7.5) containing 1 μg. (0.027 unit) of dialyzed labeled insulin was preincubated at 37°C. for five minutes with 1 ml. of heparinized diabetic or normal plasma and then incubated with pieces of rat diaphragm, or liver slices or 1 ml. of a liver homogenate. This experimental system provides an excess of degradative enzyme so that any factor tending to reduce available insulin I-131 substrate concentration will be reflected in a decreased amount of degradation products formed. At a predetermined time the reaction was terminated by pouring the incubation medium into TCA. The liver slice or quarter diaphragm was rinsed with 1 ml. of phosphate buffer, blotted with filter paper and then dissolved in potassium hydroxide. The buffer rinse was added to the incubation mixture in TCA. Subsequent processing and measurement of radioactivity in the

samples was done according to methods previously described.[11] Experiments were run in duplicate.

The results of two experiments utilizing liver slices and quarter diaphragms are shown in Figure 4. Plasma samples were selected from diabetic subjects in whom different degrees of retention of labeled insulin *in vivo* were demonstrated. Each study included separate control non-diabetic plasma. It is apparent that the greater the concentration of insulin I-131 retained in the plasma *in vivo*, the less labeled hormone was bound to and degraded by rat tissues *in vitro*.

The plasma of thirty diabetic subjects was then studied, utilizing liver homogenate suspensions. These homogenates were much more active than intact tissues in their ability to degrade labeled insulin. Hence it was possible to demonstrate more rapidly the presence of factors protecting insulin I-131 from degradation. In the presence of normal plasma about 25 per cent of the I-131 radioactivity was demonstrable in the TCA supernatant fraction (degradation products) after ten minutes of incubation. When diabetic plasma was substituted for normal plasma, degradation of the labeled insulin was depressed to varying degree, as reflected by a decrease in the TCA-supernatant. To enable comparison of individual experiments, each with its own control, the degradation of insulin I-131 in diabetic plasma is expressed as a percentage depression of the degradation taking place in normal plasma. This percentage is an index of the inhibition of degradation by diabetic plasma factors. Figure 5 illustrates the correlation between the amount of intact labeled insulin retained in the plasma *in vivo* and the depression by plasma of degradation by liver homogenate after ten minutes of incubation *in vitro*. As in the studies with liver slices and diaphragms, there is proportionately less degradation of labeled insulin *in vitro* in the plasma of diabetic subjects in whom higher concentrations of insulin I-131 are retained in the plasma *in vivo*.

Attempts to enhance or decrease plasma retention of labeled insulin *in vivo* by varying the amounts of non-labeled insulin administered preceding the test, in the manner described, met with no success. However, using the *in vitro* liver homogenate system it was possible to add relatively large amounts of unlabeled amorphous insulin. (The addition of 1 μg. of labeled insulin to 1 ml. of human plasma *in vitro* is equivalent to adding 78 units of insulin to a total plasma volume of 3,000 ml. *in vivo*.) It was reasoned that if the delayed distribution and degradation of insulin I-131 in diabetic subjects represented binding to a plasma factor, the preliminary

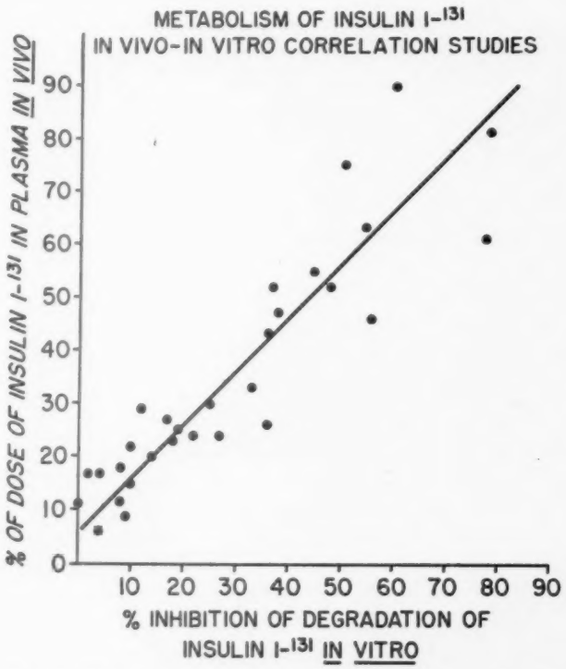

FIG. 5. The correlation between the *in vivo* retention of insulin I-131 60 minutes after injection and the inhibition of degradation of insulin I-131 by the rat liver homogenate in the presence of diabetic plasma, *in vitro*. The greater the retention *in vivo* the greater is the protective effect of diabetic plasma inhibiting degradation of the labeled hormone *in vitro*.

addition of unlabeled insulin to such plasma might serve to saturate binding sites. Therefore, labeled insulin added later would not be bound and hence could be degraded more rapidly. This hypothesis was tested with several specimens of diabetic plasma and the results are illustrated by Figure 6. Whereas the addition of amorphous insulin in increasing amounts to normal control plasma progressively depressed degradation of labeled insulin by liver homogenate, it did not depress degradation as rapidly or as uniformly in the presence of diabetic plasma. In normal plasma, added amorphous insulin apparently competed for the degradative enzyme system and hence depressed degradation of labeled insulin. However, plasma of those diabetic subjects (Nos. 100, 108 and 110),

which alone greatly inhibited the degradation of 1 μg. of labeled insulin, apparently bound up to 5 μg. of amorphous insulin and "saturated" binding sites. Here amorphous insulin seemed to compete with labeled insulin for binding sites and thus allowed insulin I-131 to remain more

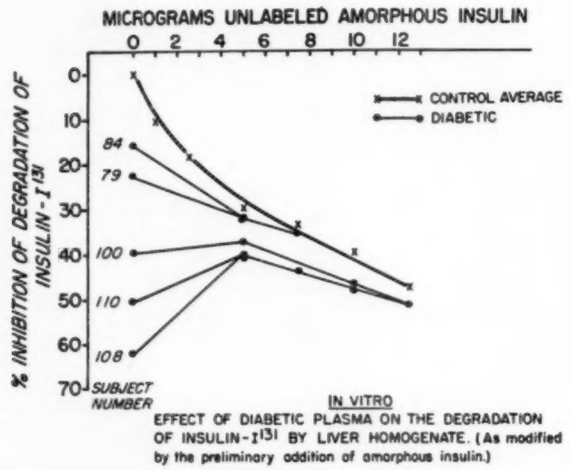

FIG. 6. Comparison of the effect of adding amorphous insulin to plasma prior to adding insulin I-131 and rat liver homogenate. The subsequent degradation of insulin I-131 in control plasma was proportionally depressed by increasing amounts of amorphous insulin. Diabetic plasma from subjects No. 84 and No. 79 initially depressed degradation of labeled insulin 16 and 23 per cent, respectively. The addition of 5 micrograms of amorphous insulin further depresses degradation, but less abruptly than in control plasma. Adding 5 μg. amorphous insulin to diabetic plasmas of subjects No. 100, 110, 108 caused an increase rather than a depression of degradation of labeled insulin. The increase in degradation with amorphous insulin is greater when diabetic plasma, which alone markedly depressed degradation of labeled insulin, is used. The graph illustrates that amorphous insulin competes with labeled insulin for plasma-binding factors. The quantity of labeled insulin used is constant, namely, 1 μg. (0.027 unit). 1 ml. plasma and 1 ml. of 1 per cent rat liver homogenate (in phosphate buffer) were used.

freely available for degradation. As illustrated, the net result was increased rather than decreased degradation of labeled insulin initially. This phenomenon was not as striking when diabetic plasma which inhibited degradation minimally (Nos. 84 and 79) was used, although depression of degradation following addition of amorphous insulin was not as rapid as in control plasma.

If a large excess of amorphous insulin (30 μg.) was added to 1 μg. of labeled insulin and incubated with plasma and liver homogenate, the depression of degradation of the labeled insulin was the same whether or not diabetic or normal plasma was used. It was only when a small amount (1 μg.) of insulin I-131 alone was used that the inhibitory effect of diabetic plasma could be demonstrated to best advantage.

Other experiments have shown that the use of either serum or oxalated or heparinized plasma from the same diabetic subject did not influence the observed depression of degradation of labeled insulin. Similarly, the ingestion of food or the administration of therapeutic insulin prior to obtaining blood samples did not influence the results. Boiling normal plasma increased its inhibitory effect on labeled insulin degradation but decreased the inhibitory effect of diabetic plasma, so that degradation was the same in both boiled normal and boiled diabetic plasma. Repeated rapid freezing and thawing of control and diabetic plasma five times in twenty-four hours did not influence the degree of depression of degradation in diabetic plasma. Dialysis of diabetic plasma for eighteen hours against phosphate buffer (pH 7.5) at 5°c. did not alter its ability to depress degradation of labeled insulin. When a diabetic person's plasma, which markedly inhibited degradation of labeled insulin *in vitro* with liver homogenate, was diluted up to 50 per cent with phosphate buffer or normal plasma, the percentage depression of degradation was slowly decreased in a linear fashion with respect to the dilution. A greater than 50 per cent dilution resulted in a rapid decrease in the inhibition of degradation but again in a linear fashion with respect to dilution.

The results of these *in vitro* experiments give correlative support to the impressions gained in the *in vivo* studies that factors intrinsic to the plasma influence distribution and degradation of the labeled hormone. This is compatible with the concept that labeled insulin is bound in some manner in the plasma, consequently it is less readily available to the tissues and is protected from enzymatic degradation. The observation that the addition of amorphous insulin does not depress degradation of labeled insulin in some diabetic plasmas to the degree observed in normal plasma lends support to the concept that in diabetic plasma there are factors which bind and compete for insulin and reduce its effective concentration. Observations of the effect of a large excess of insulin suggest that decreased degradation in the presence of diabetic plasma is the result of decreased available substrate (insulin) rather than merely

inhibition of the degradative system. The binding agent apparently is heat-labile and non-dialyzable and is not inactivated by short-term repetitive freezing and thawing. It retains its activity proportionally in dilution with buffer or normal plasma.

The demonstration of this insulin-binding factor in the plasma of many persons with diabetes was particularly interesting because none of these patients were considered to be insulin-resistant. To investigate the significance of the binding it was necessary to assay its effect on the biologic activity of insulin. Since excess amounts of insulin obscure the abnormalities observed in diabetic plasma, it was desirable to use a bio-assay method requiring a concentration of insulin no greater than that used in the *in vitro* studies. Since mice are sensitive to small amounts of insulin and have been used to demonstrate insulin-antagonizing substances in the serum of insulin-resistant diabetes, these animals were used for assay and a modification of Lowell's method[25] was employed.

BIO-ASSAY STUDIES

Plasma from persons with diabetes in whom high percentages of labeled insulin were retained *in vivo* and from normal subjects was dialyzed overnight to make it essentially sugar-free. In contrast to 0.2 ml. used by Lowell, 0.6 ml. of each plasma was mixed with 0.2 ml. of amorphous insulin solution containing 0.01 unit of insulin. These mixtures were preincubated at 37°c. for five minutes. After an initial fasting blood sample was obtained, each mouse was injected intraperitoneally with 0.8 ml. of the plasma-insulin mixture. A second blood sample was obtained forty-five minutes after the injection. Glucose determinations were made in duplicate on both the mouse blood and the dialyzed human plasma. In Experiment No. 1 the effect of intraperitoneal human plasma mixed with saline solution alone was investigated.

The results are given in Table II. The plasma of four of five diabetic subjects tested significantly inhibited the hypoglycemic effect of added amorphous insulin. Three of the four diabetic subjects had retained 90, 81 and 75 per cent of the dose of labeled insulin in the plasma at sixty minutes. The fourth subject was not tested *in vivo*. The plasma of all four subjects markedly depressed labeled insulin *in vitro*. (Figure 5.) The fifth diabetic subject had retained only 49 per cent of the labeled insulin *in vivo* and no significant inhibition of the action of insulin was noted by bio-assay. (Experiment No. 4.) The plasma-saline mixtures in Experiment No. 1 did not give rise to significant hypoglycemia or hyperglycemia. Since all the dialyzed plasma contained less than 10 mg. per cent of glucose, plasma-saline injections were not made subsequent to Experiment No. 1.

TABLE II
MOUSE INSULIN ASSAY*

	No. of Mice	Change in Blood Sugar (%)	Standard Deviation
Experiment No. 1:			
Control plasma + saline	5	−5.6	20.3
Diabetic (subject 118) plasma + saline	5	+2.9	26.5
Control plasma + insulin	5	−45.7	25.6
Diabetic (subject 119) plasma + insulin	5	−12.5	6.6
Experiment No. 2:			
Control plasma + insulin	5	−52.8	11.9
Diabetic (subject 115E) plasma + insulin	4	+9.0	7.6
Experiment No. 3:			
Control plasma + insulin	4	−74.3	32.0
Diabetic (subject 114) plasma + insulin	3	−28.7	12.7
Diabetic plasma + insulin†	4	−13.4	12.7
Experiment No. 4:			
Control plasma + insulin	4	−54.5	9.8
Diabetic (subject 113) plasma + insulin	4	−47.4	13.2

* In each experiment 0.6 ml. plasma and 0.2 ml. insulin/saline solution (0.01 unit) were injected intraperitoneally.

† *In vivo* insulin I-131 retention not measured; *in vitro* depression of degradation 70 per cent.

It is suggested from these studies that increased plasma binding implies partial biologic inactivation of insulin. This has been demonstrated with those diabetic plasma samples which show greatly increased binding *in vivo* or *in vitro*. However, it is indicated that the mouse assay may not be sufficiently sensitive to reflect the lesser abnormal binding properties demonstrated by labeled insulin studies.

COMMENTS

The evidence presented suggests that insulin I-131 is bound in the plasma of certain insulin-treated (diabetic and non-diabetic) subjects and that this binding phenomenon probably interferes with the biologic action of insulin.

The initial assumption that protein-bound radioactivity represents intact labeled insulin is open to some question. It is entirely possible that insulin undergoes a chemical change in diabetic plasma and that fragments of the molecule

bearing the radioactive label bind to plasma protein constituents and are therefore precipitable with trichloroacetic acid. Alternatively, the intact hormone molecule itself may be bound. The net result in either instance could be biologic inactivation of insulin.

The literature contains many reports of anti-insulin factors demonstrated in the plasma of insulin-resistant diabetic persons.[25–32] Bornstein[1] noted that no detectable insulin was present in the serum of such patients, using the adrenalectomized, alloxan-diabetic, hypophysectomized rat as a very insulin-sensitive animal, but it is significant that he observed no detectable insulin in the serum of many non-resistant diabetic persons. It is possible that plasma-binding of insulin, as shown by our studies, explains the lack of detectable insulin in some non-resistant diabetic subjects. Insulin may even be present in increased amounts in plasma but it may not be detectable by present methods of assay. If large amounts of exogenous insulin can be bound in the plasma of diabetic subjects it may explain the wide range of insulin tolerance and relative insulin insensitivity of the maturity-onset diabetic person. Vallance-Owen et al.[33] have recently reported that insulin added to the plasma of insulin-treated, non-ketotic but uncontrolled diabetic persons could not be recovered, as measured by glucose uptake in rat diaphragm. However, insulin added to plasma from insulin-treated diabetic persons with blood sugars within the normal range could be satisfactorily recovered in most cases. Their observations suggest that many diabetic patients require insulin to overcome an inhibitor circulating in the plasma. Plasma-binding of the hormone in insulin-treated patients might well explain these findings; it also suggests unsaturation of the binding factor in uncontrolled diabetic persons. The authors' studies did not, however, show any correlation of elevated blood sugar with the degree of plasma-binding of insulin.

Insulin is considered a weak antigen and, except in some reported cases of insulin resistance and allergic reactions of insulin,[25–32] it has been difficult to demonstrate antibodies to insulin. However, demonstration of increased plasma-binding of labeled insulin in many insulin-treated diabetic persons and non-diabetic schizophrenic subjects favors the argument that abnormal responses may reflect development of antibodies to insulin. The observation that approximately one-fifth of the diabetic patients studied do not exhibit the increased plasma-binding of insulin might be expected because of mild antigenicity and the variable response of different subjects to antigens of other types. Stress situations such as infections induce anamnestic responses in antibody production and, possibly by the same mechanism, could increase antibodies to insulin, thereby increasing insulin requirements. In some patients true insulin resistance follows extreme stress, perhaps by virtue of an exaggerated action of the same mechanism. No significant evidence has been presented that increased pituitary or adrenal activity may give rise to true insulin resistance or development of plasma-binding of insulin in non-resistant diabetic persons.

In some instances of resistance, insulin-neutralizing activity has been demonstrated in the gamma globulin fractions, and these observations have supported the concept that resistance represents antibody formation.[27,28] Since the authors' studies have not yet included detailed plasma protein fractionations, it has not been possible to show that insulin is bound to any specific component of plasma proteins in non-resistant diabetic persons. If plasma-binding does reflect antibody production, confirmation awaits demonstration of specific gamma globulin-binding and the formation of antigen-antibody complexes by standard immunologic procedures.

As there is no significant correlation between the insulin requirements of the diabetic subjects studied and the degree of plasma-binding of labeled insulin *in vivo* or *in vitro*, the therapeutic implications of these studies are not readily apparent. However, since the degree of plasma-binding appears to vary in some patients over a period of months along with variations in the diabetes or other pathologic conditions, it is possible that information of therapeutic import might be gained from repeated studies of the binding phenomenon over a longer period. If increased plasma-binding of insulin signifies partial biologic inactivation of exogenous insulin, as bio-assay studies seem to indicate, then patients with a low insulin requirement but with high plasma-binding may not be utilizing exogenous insulin and a reduction of dose or elimination of insulin might be feasible. Patients with high insulin requirements and high plasma-binding might have insulin requirements reduced by desensitization or hormone suppression of antibody formation, as is sometimes possible

in cases of insulin resistance.[34] Those patients taking large doses of insulin, yet exhibiting no increase of plasma-binding, may be receiving more insulin than they require and compensating with adrenal or pituitary anti-insulin mechanisms. More detailed studies of individual patients may serve to clarify these considerations.

SUMMARY

1. I-131 labeled insulin disappears less rapidly from the plasma of the majority of insulin-treated diabetic subjects and schizophrenic persons than from the plasma of non-diabetic persons.

2. There is also proportionately less degradation of labeled insulin by these insulin-treated subjects.

3. *In vitro* studies demonstrate the presence of a factor in plasma of insulin-treated subjects which binds labeled insulin, hinders its entry into normal rat tissues and depresses its enzymatic degradation.

4. Bio-assay studies reveal that the binding of insulin in diabetic plasma effectively reduces its hypoglycemic effect in mice.

5. It is suggested that the insulin-binding factor may be an antibody arising secondary to insulin therapy and that its presence may be of therapeutic import.

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Humoral Insulin Antagonism Associated with Diabetic Acidosis*

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THE mechanism of insulin resistance, or unusually high tolerance toward insulin, has been the subject of numerous investigations. Specific antibodies to heterologous insulins have been demonstrated in the blood of certain insulin-resistant diabetic patients.[1,2] Both clinically and experimentally, insulin resistance has been associated with increased activity of the anterior pituitary gland.[3,4] The demonstration of intracellular proteolytic enzymes exhibiting some degree of specificity toward insulin, insulinases, has led to the suggestion that excessively rapid proteolytic destruction of insulin may be a mechanism contributing to insulin resistance.[5,6]

Diabetic acidosis is almost always associated with exalted insulin requirement and tolerance. During the first twenty-four hours of treatment of patients in diabetic coma quantities of insulin are often administered which, had they been given to the same subject when not in ketosis, would have resulted in fatal hypoglycemia. This increase in tolerance toward insulin during episodes of acidosis has been attributed to adrenocortical hyperactivity, a view which finds support in the observation of an elevated concentration of cortical steroids in the blood in this condition.[7] This interpretation may be questioned, however, in view of the fact that adrenocortical stimulation has been reported to occur only late in the course of diabetic acidosis.[8] The finding of a decrease in response to insulin in dogs rendered acidotic with ammonium chloride infusion has led some workers to the conclusion that the decrease in pH of the blood may in itself be the main factor in the increased insulin tolerance in patients with diabetic acidosis.[9]

In Stadie's laboratory the procedure introduced by Gemmill[10] of measuring the increment in glycogen deposition in the rat hemidiaphragm in response to insulin has been extensively explored.[11–13] Utilizing this procedure, Marsh and Haugaard[14] have demonstrated the presence of insulin antagonist activity in the serum of a number of persons with diabetes whose chronic insulin resistance was not associated with either acidosis or infection. In one patient, reported on in detail, a decline in the titer of humoral insulin antagonist as measured by the rat hemidiaphragm technic was found to ensue several weeks after the patient's daily insulin requirement had diminished in response to a course of ACTH therapy.

The present study was undertaken to ascertain whether or not, by the hemidiaphragm technic, humoral insulin antagonist activity could be demonstrated during the acute insulin resistance associated with diabetic acidosis.

METHODS

Method of determining the insulin effect was similar to that previously reported by Stadie.[11] Male Sprague-Dawley rats weighing approximately 150 gm. were used. They were fasted for twenty-four hours prior to being killed by a blow on the head. The diaphragm was dissected with as little trauma as possible. Each hemidiaphragm was blotted and weighed; then one was equilibrated for one minute at 25°C. in a solution containing 1 ml. of serum and 1 ml. of insulin-buffer mixture so that the final insulin concentration was 0.1 unit/ml. The control hemidiaphragm was equilibrated with serum identically diluted but lacking added insulin. Alternately the right and left hemidiaphragm were exposed to insulin. Each hemidiaphragm was washed twice for thirty seconds by agitation in 10 ml. of buffer and then transferred to a stoppered flask containing 2 ml. of oxygenated buffer-glucose solution of the following composition: 0.087 M NaCl, 0.005 M $MgCl_2$, 0.040 M sodium phosphate and 0.4 per cent glucose, pH 6.8. The flasks were shaken in a water bath at 38°C. for ninety minutes. At the end of this period glycogen content of each hemi-

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TABLE 1

EFFECT OF NORMAL AND DIABETIC ACIDOSIS SERUM ON THE COMBINATION OF INSULIN AND RAT DIAPHRAGM

Source of Serum	Amount of Serum (ml.)	Insulin Effect Micromoles (Glucose Equivalent) per Gram of Tissue Mean ± SEM*		P†
No serum present	0	6.31 ± 1.71 (3)‡		.....
Normal persons	1	5.35 ± 0.48 (23)		.....
Diabetic acidosis:		During Acidosis	After Acidosis	
Case I 7/14/54	1	0.75 ± 2.22 (3)	.....	<.05
7/17/54	1	.....	1.11 ± 0.86 (3)	<.05
7/20/54	1	.....	6.60 ± 2.09 (3)	.....
Case II 10/23/54	1	3.11 ± 1.13 (5)	.....	<.05
10/25/54	1	.....	6.54 ± 0.95 (3)	.....
Case III 11/25/54	1	0.79 ± 1.43 (3)	.....	<.05
12/ 7/54	1	.....	6.25 ± 0.32 (3)	.....
Case IV 12/ 4/54	1	−0.56 ± 1.85 (4)	.....	<.02
12/ 7/54	1	.....	6.59 ± 1.02 (3)	.....
Case V 3/16/55	1	5.39 ± 0.63 (3)	.....	.....
Case VI 6/11/55	1	6.08 ± 1.11 (3)	.....	.....
Case VII 6/14/55	1	−2.62 ± 1.73 (3)	.....	<.01
6/14/55	0.5	−1.44 ± 2.34 (3)	.....	<.01
6/14/55	0.2	0.39 ± 0.87 (3)	.....	<.01
6/14/55	0.05	1.03 ± 1.32 (3)	.....	<.01
6/14/55	0.01	2.89 ± 2.17 (3)	.....	>.1
6/14/55	0.5 heated	0.65 ± 0.14 (3)	.....	<.01

* Standard error of the mean.

† When no postacidosis specimens were tested, P is based on the difference between the value found during acidosis and that obtained using normal serum.

‡ Figure in parentheses indicates number of experiments.

diaphragm was determined by Stadie's modification[15] of the method of Good, Kramer and Somogyi.[16] Glucose of the hydrolyzed glycogen was measured according to the procedure of Schales and Schales.[17] Results are expressed as micromoles of glucose per gram of wet diaphragm. Arithmetic difference in glycogen content between hemidiaphragm exposed to insulin and its control is called the insulin effect. When less than 1 ml. of serum was used during the initial equilibration period, phosphate-saline buffer was added to make the final volume 2 ml.

Serum* from patients with diabetic acidosis was obtained at the time of admission and again several days after therapy except in the patient (Case VII) who died twenty-four hours after admission. Specimens were stored frozen for as long as six months without any evident loss of activity. If the serum did not contain any insulin antagonist at the time of admission, later specimens were not tested.

Blood levels of compound F were measured according to the method of Peterson.[18]

* We are indebted to Dr. Frederick C. Goetz, University of Minnesota Medical School, for serum from Cases II, III and IV, and to Dr. Henry B. Mulholland, University of Virginia Medical School, for serum from Case VII.

RESULTS AND COMMENTS

The present evaluation of the effect of insulin alone upon the capacity of rat diaphragm tissue to accumulate glycogen is in essential agreement with earlier measurements by others.[14] In the

TABLE II

PERTINENT CLINICAL DATA ON CASES OF DIABETIC ACIDOSIS

Case, Sex Age	Date	Total Insulin on This Date	Blood Sugar (mg. %)	CO_2 (mEq./L.)	Remarks
I. F, 29....	7/14/54 7/17/54 7/20/54	2,130 525 0	560 350 52	3 27 26	Diabetes for 7 yr.; insulin started 3 wk. prior to episode of acidosis; regulated on 40 units/day; no complications of diabetes
II. F, 30....	10/23/54 10/25/54	615 100	648 250	11 28	Diabetes for 25 yr.; usually regulated on 30–60 units of insulin/day; retinopathy but no other complications
III. F, 64....	11/25/54 12/ 7/54	1,310 80	600 264	8.3	Diabetes for 5 mo.; no previous insulin; discharged on 80 units NPH/day
IV. F, 50....	12/ 4/54 12/ 7/54	230 80	200	19	Diabetes for 4 yr.; previously regulated on 25–40 units/day; discharged on 80 units/day; no complications of diabetes
V. M, 24...	3/16/55	200	346	18	Diabetes for 11 yr.; previously regulated on 120 units/day; discharged on 82 units/day; no complications
VI. F, 45....	6/11/55	230	1,025	11	Diabetes for a few weeks; no previous insulin; discharged on 35 units
VII. F, 22....	6/14/55	21,000	908	3.5	Diabetes of 8 yr.; previously regulated on 60 units/day; no complications of diabetes; peritonitis found at autopsy

absence of added serum (Table I) exposure of this tissue to insulin resulted in an increase in glucose stored as glycogen of 6.31 micromol/gm. In twenty-three determinations in which serum from one of six normal subjects was mixed with insulin, a mean insulin response of 5.35 micromol glucose/gm. tissue was found. This slight decrease, not considered significant from the present data, is still in accord with observations of others that normal serum may contain slight anti-insulin activity.[14]

The serum, secured on the day of hospitalization for acidosis, of seven patients with diabetes has been studied. In five of the seven cases 1 ml. of serum was found to contain sufficient insulin antagonist activity to diminish significantly the effect of 0.2 unit of insulin. In Case I antagonism to insulin was still demonstrable three days later but it should be noted (Table II) that on this day the patient still tolerated in excess of 500 units of insulin. Six days after admission, when the insulin requirement had fallen to zero, demonstrable insulin antagonist activity had disappeared from the serum. Disappearance of antagonist activity from the serum was observed two days after admission in Case II and three days after admission in Case IV. In each case such disappearance could be correlated with a striking decline in insulin requirement.

The serum from two patients in diabetic acidosis failed to exhibit insulin antagonist activity by the present assay. Both of these patients (Cases V and VI) were moderately responsive to insulin therapy, requiring 200 to 230 units in twenty-four hours for regulation. In Case IV, in which serum inhibitor activity was demonstrable, only 230 units of insulin were administered on the day of admission, but it may be pertinent that this patient had a relatively low initial blood sugar concentration. (Table II.)

Case VII was a patient who clinically appeared to be very refractory to insulin; 21,000 units injected in twenty-four hours failed to reduce the blood sugar concentration to hypoglycemic levels. The course and fatal outcome of this case will be described elsewhere.[19] The serum of this patient abolished the effect of 0.2 units of insulin when added in amounts as low as 0.05 ml.

Certain properties of the insulin antagonist here under consideration can be inferred from results already at hand. Activity is not destroyed by freezing. The material appears to be fairly thermostable since activity is still demonstrable after fifteen minutes of heating at 60°C. (Table I, Case VII.) In view of the exceptionally high titer subsequently discovered in this serum, it should be pointed out that the thermal destruction of 90 per cent of the activity would have escaped detection.

TABLE III
FAILURE OF SERUM FROM PATIENTS WITH UREMIC ACIDOSIS TO ANTAGONIZE INSULIN

Source of Serum	Blood pH	Serum CO_2 (mEq./L.)	Insulin Effect Micromoles (Glucose Equivalent) per Gram of Tissue Mean ± SEM*
Uremic patient	7.24	11.3	5.04 ± 1.78 (3)†
Uremic patient	7.07	12	5.43 ± 1.67 (6)
Normal serum	....	....	5.35 ± 0.48 (23)

* Standard error of the mean.
† Figure in parentheses indicates number of experiments.

That the insulin antagonist activity was not directly attributable to the lowered pH may be concluded from the fact that the serum of patients in uremic acidosis did not depress the effect of insulin. (Table III.) The fact that three days after admission, at a time when the blood CO_2 content was normal, Case I still exhibited serum insulin antagonist activity also argues against the acidity of the serum as the cause of insulin antagonism.

Consideration has been given the possibility that circulating adrenocortical steroids may be responsible for the observed effect. No inhibition of normal insulin effect could be produced by addition of compound F to normal serum prior to admixture of insulin. (Table IV.) Likewise the injection of ACTH into a patient with diabetes failed to produce inhibitory serum even though a rise in circulating compound F concentration did occur. The two patients in diabetic acidosis whose serum failed to show insulin antagonist activity exhibited marked elevations in the concentration of circulating compound F.

There did not seem to be any relation between the level of blood sugar or CO_2 on admission and the presence or absence of insulin inhibitor activity. Also, there was no correlation with the presence of complications of diabetes, duration of the disease or previous insulin therapy. In both Case III and VI the diagnosis of diabetes was made at the time of admission in acidosis. One of these patients (Case III) exhibited an insulin antagonist while the other did not. Although there was a wide range of values for the serum electrolytes on admission, there did not seem to

TABLE IV
ROLE OF ADRENAL CORTICAL HORMONES IN THE INSULIN EFFECT ON RAT DIAPHRAGM

Source of Serum	Plasma Level of Compound F* (μg. %)	Insulin Effect Micromoles (Glucose Equivalents) per Gram of Tissue Mean ± SEM†
Normal serum to which was added 100 μg. % compound F	>100	6.45 ± 1.25 (3)‡
Case V	58.2	5.39 ± 0.63 (3)
Case VI	102	6.08 ± 1.11 (3)
Diabetic patient at end of 48 hr. infusion of 200 units ACTH	40	5.18 ± 1.23 (6)
Normal serum	.....	5.35 ± 0.48 (23)

* Normal range: 8–24 μg. %
† Standard error of the mean.
‡ Figure in parentheses indicates number of experiments.

be any consistent pattern that accompanied the insulin resistance. Table II indicates that none of these patients was insulin resistant prior to the occurrence of acidosis and none of the patients who survived required excessively large doses of insulin once diabetes was regulated. Case V had had three previous episodes of diabetic acidosis requiring up to 3,300 units of insulin in the first twenty-four hours of therapy. No significance is attributed to the fact that all five patients who demonstrated insulin inhibition were women.

No studies have been carried out on the present serum to ascertain whether or not immunochemically demonstrable antibodies to insulin occurred. The rapid disappearance of insulin inhibitor activity after episodes of acidosis, noted in several patients, would argue against the present effect being due to a typical immune reaction.

The finding of a humoral insulin antagonist in the serum of patients with diabetic acidosis raises the question of the possible role which this antagonist may play in precipitating the ketosis and subsequent acidosis. The answer to this question is still entirely speculative. Studies are currently under way to characterize further the anti-insulin agent or agents. On the basis of the experiments and arguments presented we are presently inclined to discount the lowered pH

of the blood and the secretions of the adrenal cortex as major causative factors.

SUMMARY

In five of the seven patients with diabetic acidosis studied, humoral insulin antagonist activity was demonstrable by *in vitro* technics. The basis of the method employed was the measurement of extra glycogen accumulation in rat hemidiaphragm after exposure to insulin. Serum of normal subjects depressed this manifestation of insulin action little if at all, while serum from several patients with diabetic acidosis abolished the insulin effect.

A variety of observations lead to the conclusion that insulin antagonist activity is due neither to acidity nor to increased concentration of adrenocortical steroids in the blood. In several cases activity was noted to disappear within a few days after treatment of acidosis. The presence of humoral insulin inhibitor during acidosis did not appear to correlate with the level of blood glucose or CO_2, duration of diabetes, level of previous insulin requirement or presence of complications of diabetes. None of the present patients exhibited insulin resistance before or after the episode of acidosis. In general, however, there was good correlation between the amount of insulin received in the first twenty-four hours of therapy and insulin antagonist activity in the serum.

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Stable and Brittle Diabetes*

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In the early 1920's when insulin became generally available and attempts were made to obtain normoglycemic control in patients with diabetes, it was observed that in many patients such control could not be obtained. Reasons for failure were various. For example, at that time long-acting insulin preparations and antibiotics were not available. However, a few of these "poorly controlled" diabetic patients showed certain distinct characteristics, and subsequent improvements in management have not prevented extreme fluctuations in daily blood sugar values or frequent insulin reactions, or both. Some investigators were interested in studying this group of patients with diabetes and the work of Radoslav[1] and Falta,[2] and later Himsworth[3] indicated that two chief types of diabetes exist, namely, the insulin-sensitive and the insulin-insensitive types. Himsworth, by applying the glucose-insulin tolerance test, showed distinct patterns of responses to insulin in these two types of diabetes. In recent years the action of insulin in brittle diabetes has been studied by different methods;[4] furthermore, the action of glucagon in brittle diabetes has been investigated,[5–7] and modified insulin tolerance tests have been developed for the study of insulin sensitivity. Woodyatt[8] used the term 'brittle' to express the control which, for no apparent reason, breaks easily in either direction.

Recently, Lawrence[9] introduced a new classification of diabetes mellitus in which two types of patients who have diabetes with insulin insensitivity are described as (1) the lipoatrophic and (2) the lipoplethoric. In contrast to these insulin-insensitive diabetic persons, the insulin-sensitive type is described. In the present study, 'stable' is used to describe those patients with diabetes who are insulin-insensitive and 'brittle' to describe those who are insulin-sensitive. The term juvenile diabetes is not used because in this work only adults with brittle diabetes have been studied.

In addition to insulin sensitivity, patients with brittle diabetes have another distinct metabolic feature. Ketosis rapidly and repeatedly develops despite treatment with insulin to attempt to establish normoglycemic control. If adequate insulin is not administered, diabetic coma will develop in these patients. Ketosis indicates increased catabolism of fat. On the basis of this finding one may postulate that fat is utilized in subjects with brittle diabetes in a different pattern or at a different rate from that in subjects with stable diabetes. If such metabolic differences in carbohydrate and fat are present in patients with brittle diabetes, one is justified in accepting the possibility that this type of diabetes is a separate metabolic entity.

The present study was undertaken to ascertain not only whether or not other clinical or biochemical features distinguish brittle from stable diabetes but also whether or not the incidence of late degenerative complications is different in these two groups of patients.

MATERIALS AND METHODS

Selection of Patients. The patients included in this study were followed at the diabetic outpatient department for a number of years. They were selected during a period of one year on the following basis: (1) They were not less than twenty-five years of age, and (2) not less than sixty blood determinations were obtained during several years of follow-up, with an average of fifteen to twenty-five determinations per year.

Only adult patients were selected for this study for several reasons. It has been found that the brittle type of diabetes frequently is seen in young patients. It is not known what portion of these patients have true insulin sensitivity and how many have brittle diabetes resulting from inconsistency of physical exercise. The authors believe that on clinical grounds alone, or with the classification used in this study, accurate separation of these two categories would be impossible. Most interest was devoted to studying the age group in which late degenerative complications are prone to develop. Another reason for not including young diabetic persons was the belief that unhomogeneous

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metabolic conditions, in a broader sense, should not be compared, as it has been shown in experimental animal work[10] that a close relationship exists between growth processes and carbohydrate metabolism. For this reason there is some doubt as to whether or not impaired glucose homeostasis, which clinically seems to be the same in adult and young diabetic persons, really reflects the same metabolic condition.

Another limiting factor in the selection of patients was the number of blood sugar determinations performed over a period of years in each patient. A small number of blood sugar tests would introduce a significant error in calculation of the percentage values.

The routine re-examination of patients with diabetes at this outpatient department has for more than thirty years been as follows: Diabetic patients are rechecked two to four times yearly and for one or more consecutive days during each visit. Control of diabetes is evaluated partly on the basis of blood sugar levels estimated two or three times daily (at 7 A.M., 11 A.M. and 4 P.M.) and repeated urinalysis for sugar and ketone bodies. Meals for these diabetic persons are served in the dining room of the outpatient department and diets are under the supervision of the attending physician and dietitian. All patients are receiving insulin and are on weighed or measured diets.

Patients with diabetes who have hyperthyroidism or disease of the gastrointestinal tract, pancreas or liver were excluded from this study since those conditions are known to have a bearing on the stability of their control. Patients with diabetes of anterior pituitary or adrenal origin also were excluded.

Classification of Patients. An attempt has been made to apply objective criteria in classifying the patients with diabetes under study into two groups, stable and brittle. An insulin tolerance test was not used because it was impractical for the large number of patients studied. The frequency of insulin reactions being subjective information given by patients, it was believed not to be a sufficient criterion for classification. The authors wish to emphasize that in evaluating this classification one should bear in mind the aim has been to obtain a normoglycemic control in all these patients for the period they have been followed. Changes in the dose of insulin, in the insulin preparations employed in the diets and in the available glucose distribution have been repeatedly introduced in attempting such control.

The criteria have been designed to express objectively the characteristic blood sugar pattern noted in patients with brittle diabetes: extreme fluctuations of blood sugar values that are observed consistently for years, especially when two or three determinations are performed daily and at fairly frequent intervals.

The first criterion has been outlined by obtaining "the per cent distribution curve of the over-all blood sugar determinations." It is obtained by the following procedure. Four arbitrary blood sugar ranges are used. The first range includes blood sugar values less than 120 mg. per cent; the second is from 120 to 150 mg. per cent; the third from 150 to 200 mg. per cent; and the fourth above 200 mg. per cent. All blood sugar levels of each patient are distributed according to their value in one of these ranges. An example is given for simplification. A patient has had, in an interval of eight years, 123 blood sugar determinations. When these are distributed according to their value in the four ranges presented above, one obtains the following: In the first range, twenty-nine tests; in the second range, fifteen; in the third range, sixteen; and in the fourth range, sixty-three. The per cent values for the figures in each range respectively are: 23, 12, 13 and 51 per cent. These percentages express the frequency of distribution of the patient's blood sugar levels for an interval of eight years, of which 23 per cent fell in the normal range, smaller percentages in the ranges between 120 to 150 mg. per cent and 150 to 200 mg. per cent, and the highest percentage in the highest blood sugar range. When these percentages are plotted against the blood sugar ranges, a distribution curve is obtained. It is apparent that in such an expression the time relationship is taken into consideration only roughly; but by pooling the figures and obtaining the percentage distribution curve in each patient, one has a more objective impression of the over-all type of control for the entire study period.

The second criterion is based on the extremes of difference between the two or three blood sugar values obtained in a single day and for repeated consecutive days. Three ranges of differences have been used: One for differences above 100 mg. per cent, a second for differences between 50 to 100 mg. per cent, and a third for differences less than 50 mg. per cent. The daily differences for each patient have been distributed according to their value in the three ranges and the percentages for each group calculated. It is apparent that in this criterion the daily time relationship of blood sugars has been taken into consideration. For convenience this is called "the per cent frequency of the differences on repeated daily blood sugars."

For the third criterion the incidences of blood sugar values below 60 mg. per cent and above 300 mg. per cent are taken into consideration, and the per cent value from the total number of determinations is calculated for each patient. This is called "the per cent frequency of the extreme blood sugar values."

The fourth criterion has been based on the presence or absence (on repeated tests) of 2 plus to 3 plus ketone bodies in the urine, as determined by the bedside technic. This test was carried out during the usual recheck periods, and only the tests performed at these times were evaluated. Tests done during the period in which the patient was first seen and the period in which the patient was hospitalized for diabetic coma were not taken into consideration.

Studies Performed. History charts for all patients were reviewed and notes were made on previous symptoms, physical findings and previous diagnoses. Also, the patients were interviewed and specifically questioned in regard to symptoms of heart disease, frequency of insulin reactions, adherence to meal

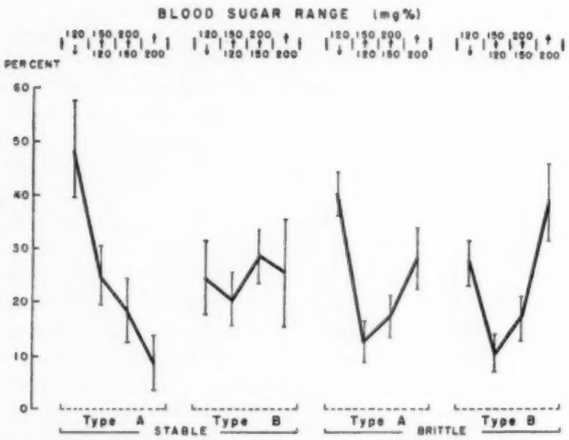

FIG. 1. The per cent distribution curve of the over-all blood sugar determinations (percentages express frequency of distribution).

schedules, and sensitivity to physical exertion. Weight of the patient was taken into consideration and notations made for weight when last seen, maximum weight attained during life, and differences of these and the ideal weights.*

All patients had had a physical examination during the preceding year. Renal function was evaluated on repeated urinalyses which were obtained at each follow-up period, and the presence of proteinuria noted. Blood urea determinations and, in a large number of patients, urea clearance tests were performed. In a few cases Addis' tests were done. For evaluation of the degree of retinopathy all patients were examined after mydriasis by the same ophthalmologist. The condition of the cardiovascular system was evaluated on the basis of the history, physical findings, electrocardiogram, roentgenogram of the heart and repeated blood pressure readings. In a large number of patients from each group who were under diabetic control and in a fasting condition, blood specimens were taken for the study of ultracentrifugal lipoproteins, Tiselius electrophoretic patterns and blood cholesterol determinations. The results of these tests will be presented in another publication. In a number of patients from each group intravenous glucagon tests were performed and the effect on the concentration of blood glucose, ketone bodies and lipoproteins were studied.[7]

* For ideal weights the tables of the Association of Life Insurance Directors and Actuarial Society of America were used.[11]

RESULTS

Classification. A total of 172 patients was studied of which 149 fulfilled all the criteria for classification and were separated into two groups, stable and brittle. Twenty-three patients did not fulfill all criteria and will be discussed as a separate group.

In Figure 1 the per cent distribution curves of the over-all blood sugar values are plotted for patients with stable and brittle diabetes according to the method described previously. Of seventy-nine patients with stable diabetes, forty-five gave type A curves and thirty-four type B. In the type A curve, the majority (over 60 per cent) of the tests lay in the two lower and most normal ranges. In the type B curve, less than 60 per cent lay in the two most normal ranges. The shape of the type A curve for patients with stable diabetes is a descending, almost straight line, with a high per cent frequency of blood sugars in the lower ranges and consecutive lower percentages for the higher blood sugar ranges. The mean per cent value for type A in patients with stable diabetes is, for the first range, 48.2 per cent (S. D. ± 9.7); for the second range 24.6 per cent (S. D. ± 6.8); for the third range 18.1 per cent (S. D. ± 6.7); and for the fourth range 8.3 per cent (S. D. ± 5.3). The type B curve for patients with stable diabetes is distributed around the 25 per cent level for all the blood sugar ranges. The mean per cent value for type B in patients with stable diabetes is, for the first range, 24.9 per cent (S. D. ± 8.0); for the second range 20.5 per cent (S. D. ± 6.3); for the third range 28.5 per cent (S. D. ± 6.9); and for the fourth range 25.4 per cent (S. D. ± 11.4). Thus the type A curve represents more complete control of blood sugar levels, and the type B curve less complete.

In seventy patients with brittle diabetes a V-shaped curve is obtained indicating that these patients have a high per cent frequency of blood sugar values of the two extreme ranges, the very low (less than 120 mg. per cent) and the very high (above 200 mg. per cent). Two types of V-shaped curves are obtained, type A (thirty-five cases) in which the per cent value for the first range is higher than the per cent value of the last range, and type B (thirty-five cases) in which this is reversed. The mean per cent value for type A in patients with brittle diabetes is, for the first range, 40.6 per cent (S. D. ± 4.0); for the second range 12.6 per cent (S. D. ± 3.8); for the third

range 17.5 per cent (S. D. ± 4.4); and for the fourth range 28.2 per cent (S. D. ± 5.7). The mean per cent value for type B in brittle diabetics is, for the first range, 27.8 per cent (S. D. ± 4.7); for the second range 10.5 per cent (S. D. ± 3.5); for the third range 16.9 per cent (S. D. ± 4.1); and for the fourth range 43.9 per cent (S. D. ± 7.1).

In a few patients with brittle diabetes who have had a large number of blood sugar determinations, over a period of years, two further analyses of their distribution curves were made in order to evaluate other factors that possibly could influence the form of these curves. The blood sugar values were separated into fasting and non-fasting, and the distribution curves for these were compared. It was of interest to note that in the majority of cases studied the V-shaped distribution curves in patients with brittle diabetes for the fasting and non-fasting blood sugars were quite identical. This would indicate that the V-shaped curves obtained when fasting and non-fasting blood sugar values are pooled do not result from low fasting blood sugars as might have been expected, taking into consideration the fact that most of the patients with brittle diabetes were receiving protamine zinc insulin. In some cases of brittle diabetes, with a follow-up study of twelve to twenty years and a total of 300 to 400 blood sugar determinations, separate distribution curves were obtained for four- to five-year intervals. These curves were consistently of the V-shaped form and of almost the same per cent distribution for each patient. This indicates that in these patients the response to insulin, in the attempt to obtain a normoglycemic control, has been consistently the same during all the years of follow-up. No detailed data are given for these studies as the number of cases with such a long follow-up and such a large number of blood sugar determinations was small.

Figure 2 presents the per cent values for the daily differences above 100 mg. per cent, between 50 and 100 mg. per cent, and below 50 mg. per cent in the two diabetic groups. It will be seen that in all cases of stable diabetes there is a high percentage of fluctuation, less than 50 mg. per cent in a day.

For daily differences above 100 mg. per cent the mean per cents in brittle diabetes are 25 per cent (S. D. ± 7.9) and 30.8 per cent (S. D. ± 7.9), as compared with stable diabetes which are 5.3 per cent (S. D. ± 4.9) and 8.3 per cent (S. D. ± 5.6). For differences below 50 mg. per cent, patients with brittle diabetes showed lower mean per cent values: 40.3 per cent (S. D. ± 9.3) and 35.6 per cent (S. D. ± 10.6) as compared with 70.8 per cent (S. D. ± 13.1) and 63.3 per cent (S. D. ± 12.2) for patients with stable diabetes.

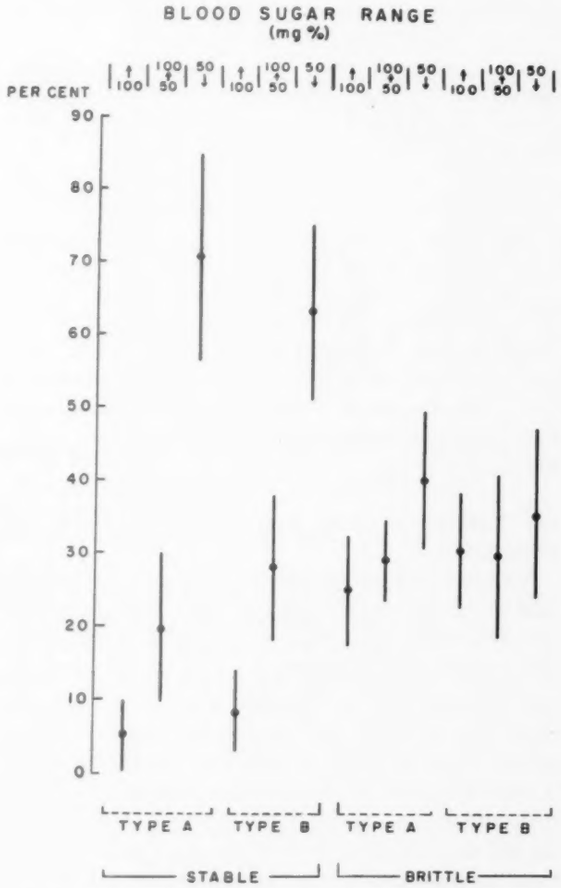

FIG. 2. The per cent frequency of the differences on repeated daily blood sugars.

Figure 3 records the per cent incidence of extreme blood sugars found in the two diabetic groups. Patients with brittle diabetes showed, for blood sugars below 60 mg. per cent, mean values of 10.8 per cent and 10.6 per cent as compared with mean values of 1.1 per cent and 0.8 per cent for patients with stable diabetes. For the per cent incidence of blood sugar values above 300 mg. per cent, no significant differences were found between the two types of diabetes.

In summary, the characteristics of the group classified as having brittle diabetes were as follows: (1) V-shaped distribution curve with high percentages in the extremely low and the

extremely high blood sugar ranges as compared with low percentages in the intermediate ranges; (2) incidence of 15 per cent or more of occurrence of daily differences above 100 mg. per cent; (3) incidence of 5 per cent and over in blood sugar values below 60 mg. per cent;

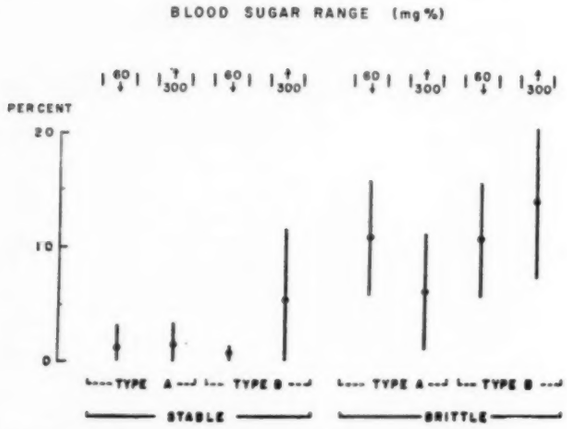

FIG. 3. The per cent frequency of the extreme blood sugar values.

(4) consistent and frequent appearance of ketonuria.

The criteria under which patients were classified as having stable diabetes are the following: (1) If the distribution curve was not V-shaped, the patients were classified as stable. They were included in type A if the addition of the per cent value for the first and second ranges was above 60 per cent and type B when below 60 per cent. Type A curve in stable diabetes is almost a descending line and type B shows a distribution for all the ranges at the 25 per cent level. In this type, in most cases, the higher per cent value was obtained in the third blood sugar range. (2) Below a 15 per cent incidence for the range of daily differences above 100 mg. per cent. (3) Below a 5 per cent incidence for blood sugar values less than 60 mg. per cent. (4) The absence of consistently repeated 2 or 3 plus ketonuria.

After classifying these patients as having stable or brittle diabetes, on the basis of these objective data, the relationship of these groups to the frequency of insulin reaction was studied. An attempt was made to express the frequency of insulin reactions in a semi-quantitative way based on the record history and the information obtained by questioning each patient. This classification is presented in Table I. The data indicate the frequency of insulin reactions occurring for a period of several years and not only of the last year. In certain instances in which the frequency of insulin reactions changed, intermediate groups were used.

In Figure 4 the frequency of insulin reactions is recorded percentagewise for each grade of Table I and a comparison is made between the

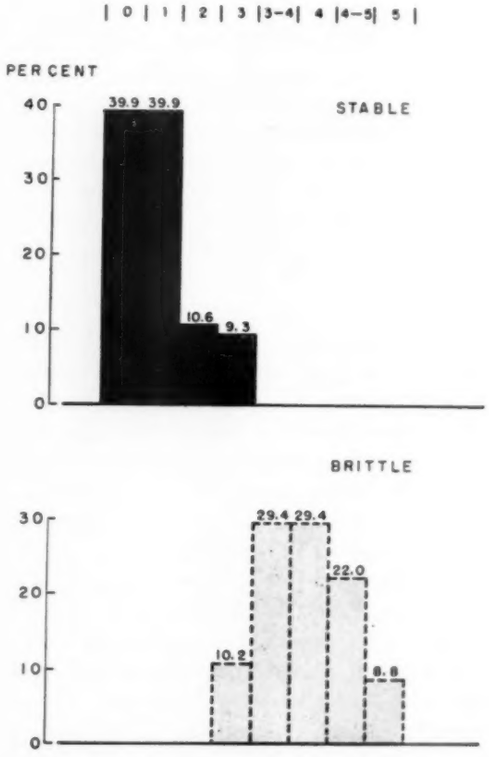

FIG. 4. Grade of frequency of insulin reactions in stable and brittle diabetic patients.

two diabetic groups. It is apparent that a clear-cut distinction between the different grades of frequency of insulin reactions is not feasible; there is an overlap between stable and brittle diabetes in respect to the frequency of insulin

TABLE I
GRADES OF FREQUENCY OF INSULIN REACTIONS

Grade	Frequency of Insulin Reactions
0	None
1+	One or two since diabetes
2+	One or two yearly (consistently)
3+	One monthly (consistently)
3–4+	Between grades 3+ and 4+ (not consistently)
4+	One weekly (consistently)
4–5+	Between grades 4+ and 5+ (not consistently)
5±	Two or three weekly, or more

TABLE II

ATYPICAL CASES—GROUP I

Diabetic Patients with Typical Findings in the First Criterion and Atypical Findings in Other Criteria

Case No. and Patient's Initials	First Criterion (distribution curve)	Second Criterion (differences above 100 mg. %)	Third Criterion (differences below 60 mg. %)	Fourth Criterion (ketonuria)	Grade of Frequency of Insulin Reactions
1, W. P.	T*	A†	A	0	3+
2, J. H.	T	T	A	0	2+
3, E. A.	T	T	A	+	3+
4, T. V.	T	A	T	0	0
5, E. L.	T	T	T	0	1+
6, F. T.	T	A	A	+	3+
7, L. B.	T	A	A	0	1+
8, B. G.	T	T	A	0	2+
9, M. S.	T	T	A	0	4–5+
10, C. B.	T	T	T	0	3+
11, I. C.	T	A	A	0	0
12, M. P.	T	T	A	0	0

* T = typical findings.
† A = atypical findings.

TABLE III

ATYPICAL CASES—GROUP II

Diabetic Patients with Typical Findings in the Second Criterion and Atypical Findings in Other Criteria

Case No. and Patient's Initials	First Criterion (distribution curve)	Second Criterion (differences above 100 mg. %)	Third Criterion (differences below 60 mg. %)	Fourth Criterion (ketonuria)	Grade of Frequency of Insulin Reactions
1, R. R.	A†	T*	A	0	3–4+
2, A. M.	A	T	T	+	3+
3, H. E.	A	T	T	0	3–4+
4, R. C.	A	T	T	+	4+
5, J. S.	A	T	A	0	4+
6, E. M.	A	T	T	0	3+
7, C. S.	A	T	A	0	3+
8, I. S.	A	T	A	+	3+
9, A. B.	A	T	A	+	3+
10, J. Y.	A	T	A	0	4+
11, E. V.	A	T	T	+	3–4+

* T = typical findings.
† A = atypical findings.

reactions seen in grade 3. If one is aware of the difficulty in separating grade 3 from grade 3–4, it should be apparent that if the classification were based on the frequency of insulin reactions alone almost 30 per cent of patients with brittle diabetes could be classified as stable, or 10 per cent of patients with stable diabetes as brittle.

Of 172 patients studied, twenty-three patients did not meet all criteria for classification as having stable or brittle diabetes. These atypical cases were divided further into two groups. The first group (Table II, atypical group I) includes those patients who showed a typical V-shaped curve but were atypical in respect to one, two or three of the other criteria. In this group of patients only two of twelve showed ketonuria, and only one had quite frequent insulin reactions; three never had had any and seven had had infrequent insulin reactions. The second group includes those patients (Table III, atypical group II) who did not show typical V-shaped curves but

in all cases the differences above 100 mg. per cent were above 15 per cent, as seen in patients with brittle diabetes. It was interesting to note that in this group there was a higher frequency of insulin reactions and ketonuria as compared with the previous group. On this basis this group could be classified as "possibly brittle."

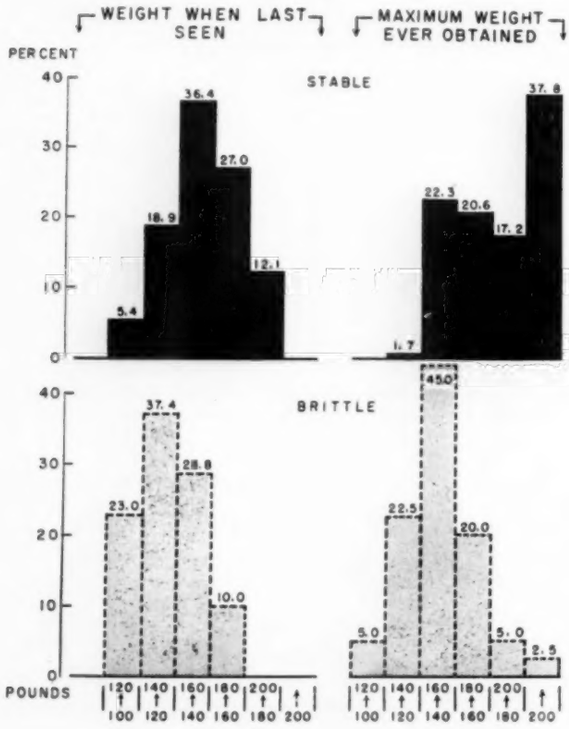

FIG. 5.

Clinical Data. Clinical data of the cases that fulfilled all the criteria of the classification shall be presented first.

Of seventy-nine patients with stable diabetes all were more than forty years of age except two (range thirty-six to eighty-three years); and of seventy patients with brittle diabetes fifty-three were more than forty years of age (range forty-one to eighty-four years) and seventeen between the ages of twenty-five and thirty years.

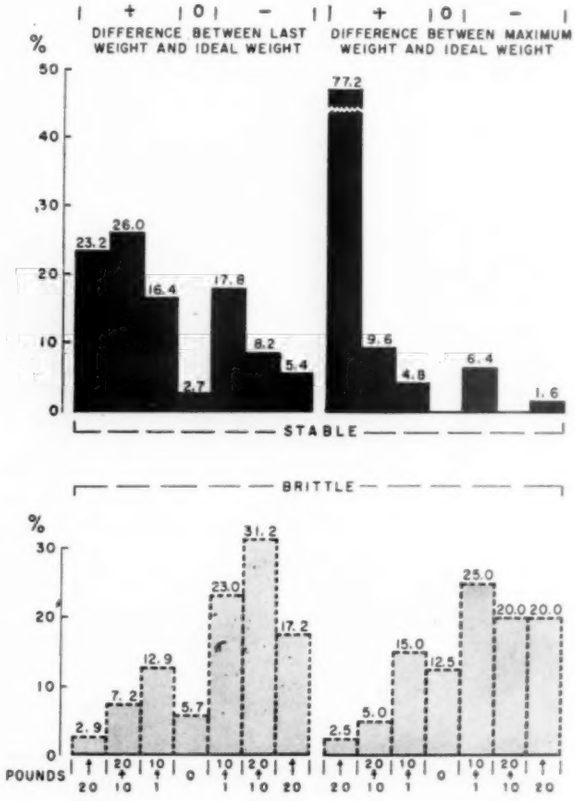

FIG. 6.

Of the patients with stable diabetes forty-three were women and thirty-six were men: a ratio of females to males of 1.19:1. Of the patients with brittle diabetes forty-four were women and twenty-six were men: a ratio of females to males of 1.69:1.

The duration of diabetes since first diagnosed up to the time the patient was last seen is recorded in Table IV.

TABLE IV
DURATION OF DIABETES IN PATIENTS STUDIED

Duration of Diabetes (yr.)	Stable		Brittle	
	No. of Patients	Range (yr.)	No. of Patients	Range (yr.)
Above 30	1	10 to 37	2	10 to 33
20 to 30	19		11	
10 to 20	44		35	
1 to 10	15	4 to 8	22	2 to 8

In Figure 5 the weights of patients when last seen are recorded. In stable diabetes a higher incidence of patients weighing between 140 and 160 pounds was found as compared with the highest percentage in cases of brittle diabetes in the group weighing 120 to 140 pounds. For their maximum weights the highest percentage was found for patients with stable diabetes in the group weighing more than 200 pounds, as compared with cases of brittle diabetes in which the

highest percentage was present in the group weighing 140 to 160 pounds. By comparing the present weights and maximum weights of these patients to their ideal weights, it is noted (Fig. 6, Tables V and VI) that 91.6 per cent of patients with stable diabetes have had a maximum weight above their ideal weight as compared with 65.0 per cent of patients with brittle diabetes. Patients with stable diabetes showed a 77.2 per cent incidence of increased weights of 20 pounds or more above their ideal weight as compared with 20 per cent in cases of brittle diabetes. When the present weights were compared with the ideal weights, 65.6 per cent of patients who had stable diabetes showed weights above ideal as compared with 23 per cent in patients who had brittle diabetes. In cases of stable diabetes 23.2 per cent of the patients showed differences of 20 pounds or more as compared with 2.9 per cent in patients with brittle diabetes.

TABLE V
DIFFERENCE BETWEEN WEIGHTS WHEN LAST SEEN AND IDEAL WEIGHTS

	Stable (%)	Brittle (%)
Above ideal weight:		
Difference of 20 pounds and more	23.2	2.9
Difference of 10 to 20 pounds	26.0	7.2
Difference of 1 to 10 pounds	16.4	12.9
Total	65.6	23.0
Equal to ideal weight:	2.7	5.7
Below ideal weight:		
Difference of 20 pounds and more	5.4	17.2
Difference of 10 to 20 pounds	8.2	31.2
Difference of 1 to 10 pounds	17.8	23.0
Total	31.4	71.4

TABLE VI
DIFFERENCE BETWEEN MAXIMUM WEIGHTS AND IDEAL WEIGHTS

	Stable (%)	Brittle (%)
Above ideal weight:		
Difference of 20 pounds and more	77.2	20.0
Difference of 10 to 20 pounds	9.6	20.0
Difference of 1 to 10 pounds	4.8	25.0
Total	91.6	65.0
Equal to ideal weight:	....	12.5
Below ideal weight:		
Difference of 20 pounds and more	1.6	2.5
Difference of 10 to 20 pounds	....	5.0
Difference of 1 to 10 pounds	6.4	15.0
Total	8.0	22.5

Frequency of Insulin Reactions. Several types of insulin preparations and their combinations have been used in attempting to obtain normoglycemic control in these patients. At least two types of preparations were administered at one time in 89.6 per cent of patients with brittle diabetes and in 48 per cent of patients with stable diabetes. In about 20 per cent of patients in both groups, three or four types of insulin preparations have been tried and their control evaluated. Most patients with brittle diabetes were treated with a mixture of protamine zinc and regular insulin. Patients with stable diabetes, in contrast, were mostly treated with protamine insulin alone. Small supplementary insulin doses were used, depending upon the results of daily urine sugar tests, and 67.6 per cent of the patients with brittle diabetes used these consistently. In patients with stable diabetes 27 per cent used these supplementary doses inconsistently and 72.9 per cent did not use them at all. A classification for frequency of insulin reactions has already been presented. In 60 per cent of patients with brittle diabetes insulin reactions occurred from one to two or three times weekly. Forty per cent of the patients with stable diabetes never had any insulin reactions. The overlap seen in the two diabetic groups has already been discussed.

The relationship of the occurrence of hypoglycemic reactions to physical exercise was noted. In patients with brittle diabetes 86 per cent appeared to be sensitive to physical work; in 87.2 per cent of patients with stable diabetes this never occurred.

Insulogenic Lipodystrophies. In both diabetic groups the incidence of insulogenic lipodystrophies was noted. These were present in 29 per cent of the patients with brittle diabetes; none were found in patients with stable diabetes. The type of lipodystrophy seen in these patients was that designated insulin lipoatrophy. The highest incidence of lipoatrophy was present in female patients with diabetes.

TABLE VII

INCIDENCE OF DIABETIC RETINOPATHY

(Duration of Diabetes, More than 10 Years)

Diabetes	No. of Patients	Grade of Diabetic Retinopathy									
		0		I		II		III		IV	
		No.	%	No.	%	No.	%	No.	%	No.	%
Stable:											
Type A.....	32	15	46.8	8	24.9	7	21.8	1	3.1	1	3.1
Type B.....	27	11	40.7	0	....	6	22.2	3	11.1	7	25.9
Total.....	59	26	43.8	8	13.5	13	21.9	4	6.6	8	13.4
Brittle:											
Type A.....	25	19	76.0	4	16.0	1	4.0	0	....	1	4.0
Type B.....	20	8	40.0	7	35.0	5	25.0	0	....	0	....
Total.....	45	27	59.9	11	24.2	6	13.3	0	....	1	2.2

Degenerative Complications. Only those patients with a complete history have been included in the study.

Diabetic retinopathy: The following classification of diabetic retinopathy was used:[12–14] Grade I, presence of punctate hemorrhages or microaneurysms alone; grade II, presence of punctate hemorrhages or capillary aneurysms, scattered hemorrhages of larger size, and small, hard, white or yellow exudates; grade III, presence of punctate hemorrhages or capillary aneurysms, small, hard, white or yellow exudates, scattered large hemorrhages and neovascularization; grade IV, presence of the findings listed under grade III plus retinitis proliferans or retinal detachment. The same ophthalmologist evaluated the grades of retinopathy in all patients.

In patients with diabetes for less than ten years, the incidence of diabetic retinopathy was as follows: of thirteen patients with stable diabetes eight did not have any diabetic retinopathy, four had grade I and one grade II. Of twenty-two persons with brittle diabetes nineteen did not have any diabetic retinopathy and three had grade I. Due to the smaller number of cases in this group the percentages were not computed.

In Table VII the per cent incidences of the various grades of diabetic retinopathy are recorded for the different types of diabetes in those patients who have had diabetes for more than ten years. The incidence of diabetic retinopathy is much higher in patients with stable diabetes than in those with brittle diabetes; 20.0 per cent of the patients in the stable group had diabetic retinopathy grade III or IV, as compared with 2.2 per cent in the brittle group. In the latter group, six patients who had had diabetes for twenty-one, twenty-three, twenty-five, twenty-six, twenty-nine and twenty-nine years, respectively, had no diabetic retinopathy, and only one patient with brittle diabetes who had had the disease for thirty-three years had diabetic retinopathy grade IV. The latter patient, when examined in the Department of Ophthalmology two years earlier (duration of diabetes thirty-one years), showed no signs of retinopathy. If types A and B in stable diabetes (Table VII) are compared, it will be noted that the incidence of diabetic retinopathy is much higher in type B (that is, in the group with higher blood sugar levels). The incidence of retinopathy in the two types of patients with brittle diabetes are not distinctly different. In Figure 7 the per cent incidence of diabetic retinopathy is plotted when computed for the total number of patients in each group.

Diabetic nephropathy: Repeated urinalyses were of course obtained in all patients, and urea clearance rates were determined in 50 per cent of the group with stable diabetes. Of seventy-nine patients with stable diabetes, three showed

TABLE VIII
INCIDENCE OF CARDIOVASCULAR COMPLICATIONS
(Duration of Diabetes, More than 10 Years)

Diabetes	No. of Patients	O		H		A	
		No.	%	No.	%	No.	%
Stable:							
Type A....	33	14	42.4	10	30.3	9	27.2
Type B....	25	4	16.0	6	24.0	15	60.0
Total..	58	18	30.9	16	27.5	24	72.2
Brittle:							
Type A....	26	16	61.4	5	19.2	5	19.2
Type B....	21	17	80.9	3	14.2	1	4.7
Total..	47	33	69.9	8	16.9	6	12.7

Note: O = cardiovascular system normal; H = hypertension present; A = arteriosclerosis present.

evidence of renal impairment. None of the sixty-nine patients with brittle diabetes showed any proteinuria other than occasional traces. In fifty of these patients urea clearance tests were performed and were found to be normal.

Degenerative lesions in cardiovascular system: These were divided into three groups: The first group (O) includes patients with no clinical or laboratory evidence of arteriosclerosis. The second group (H) includes those with hypertension but no other evidence of cardiovascular damage. A diagnosis of hypertension was made, according to the criteria of the New York Heart Association, when on repeated recordings a systolic blood pressure is over 140 mm. Hg or a diastolic over 90 mm. Hg; these values are considered the lowest limit for the diagnosis of high blood pressure. The third group (A) includes all patients who presented several indications of arteriosclerosis (angina pectoris, calcified arteries, electrocardiographic findings of myocardial infarction, or conduction disturbances, evidence of impaired peripheral circulation, funduscopic findings); in most of these patients diagnosis had been made previously by the physician in charge. In patients with diabetes of less than ten years' duration the incidence of complications in the cardiovascular system were as follows: of fourteen patients with stable diabetes three had hypertension only and seven had overt arteriosclerosis; of twenty-one patients with brittle diabetes only two had overt arteriosclerosis.

In Table VIII the per cent incidence of arteriosclerotic findings are recorded for cases of stable and brittle diabetes of more than ten years' duration. Patients with stable diabetes showed a higher incidence (72.2 per cent) of degenerative

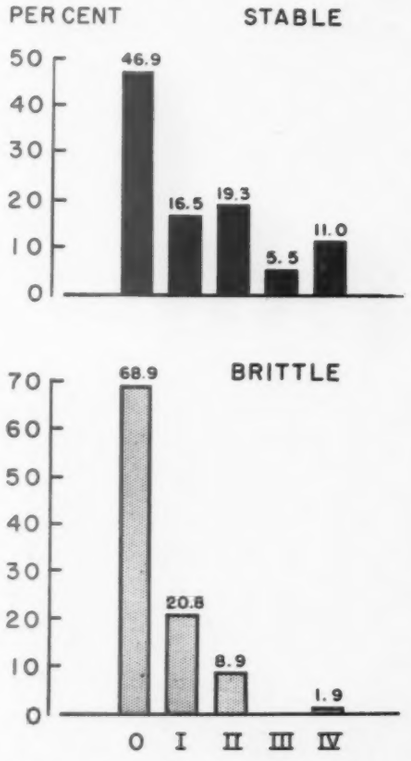

FIG. 7. Grade of diabetic retinopathy.

lesions in the cardiovascular system than did those with brittle diabetes (12.7 per cent). There was a marked difference between type A and type B in patients with stable diabetes, the higher incidence being in type B (60.0 per cent) as compared with type A (27.2 per cent).

Due to the higher female-to-male ratio in brittle diabetes the incidence of cardiovascular damage was computed according to sex for patients having diabetes for more than ten years. Twenty-four men and thirty-four women had stable diabetes; eight of the men (33.2 per cent) and sixteen of the women (47.0 per cent) had overt arteriosclerosis. Nineteen men and twenty-eight women had brittle diabetes; two of the men (11.5 per cent) and four of the women (14.2 per cent) had overt arteriosclerosis. These figures indicate that the lower incidence of frank arteriosclerosis found in brittle diabetes is not the result of the higher female-to-male ratio observed in this group.

TABLE IX

CLINICAL FINDINGS IN DIABETIC PATIENTS OF ATYPICAL GROUP I

Case No. and Patient's Initials	Age and Sex	Duration of Diabetes (yr.)	Weight (lb.)	Maximum Weight (lb.)	Difference between Last Weight and Ideal Weight	Difference between Maximum Weight and Ideal Weight	Insulin Lipodystrophies	Retinopathy (grade)	Nephropathy	Arteriosclerosis
1, W. P.	65, M	37	157	162	−17	−5	0	IV	0	A
2, J. H.	63, F	15	142	...	−2	....	0	I	+	A
3, E. A.	33, F	8	146	165	+13	+32	+	I	0	O
4, T. V.	80, F	26	153	...	+22	....	0	II	0	A
5, E. L.	56, M	20	130	165	−9	+26	0	I	0	O
6, F. T.	39, M	10	152	165	−1	+12	0	I	0	O
7, L. B.	69, F	14	108	180	−22	+50	0	0	0	A
8, B. G.	31, M	21	146	...	+14	....	0	IV	+	A
9, M. S.	29, M	14	160	...	−10	....	0	III	+	A
10, C. B.	52, M	21	129	...	−47	....	0	IV	+	O
11, I. C.	65, F	31	158	200	+14	+56	0	IV	0	O
12, M. P.	81, F	13	118	...	−6	....	0	0	0	A

TABLE X

CLINICAL FINDINGS IN DIABETIC PATIENTS OF ATYPICAL GROUP II

Case No. and Patient's Initials	Age and Sex	Duration of Diabetes (yr.)	Weight (lb.)	Maximum Weight (lb.)	Difference between Last Weight and Ideal Weight	Difference between Maximum Weight and Ideal Weight	Insulin Lipodystrophies	Retinopathy (grade)	Nephropathy	Arteriosclerosis
1, R. R.	48, F	13	129	151	−21	+1	0	0	0	A
2, A. M.	33, F	11	161	162	+3	+1	0	0	0	O
3, H. E.	37, M	17	135	183	−14	+34	0	0	0	O
4, R. C.	64, M	12	133	165	−9	+23	+	0	0	O
5, J. S.	52, M	12	120	145	−10	+2	0	0	0	O
6, E. M.	28, M	10	134	...	−10	....	+	0	0	O
7, C. S.	74, M	23	148	235	+10	+97	0	I	0	A
8, I. S.	43, M	17	165	185	+20	+37	0	II	0	O
9, A. B.	34, F	16	122	...	0	....	0	I	0	O
10, J. Y.	47, F	15	132	140	−4	+8	+	II	0	H
11, E. V.	44, M	10	164	...	+17	....	0	0	0	A

A further analysis, of relation to age of patient, was made of the incidence of overt arteriosclerosis in patients with diabetes for more than ten years. It was noted that in persons with stable diabetes the higher incidence of arteriosclerosis occurred in the range of sixty-one to seventy years (thirteen of twenty-four patients with overt arteriosclerosis) as compared with patients with brittle diabetes for the same age range (two of six with overt arteriosclerosis). In view of the higher incidence of patients included in the age range of sixty-one to seventy years in the stable diabetic group (50.4 per cent) as compared with the brittle diabetic group (14.2 per cent), it should be indicated that this difference may have a definite bearing on the higher incidence of arteriosclerosis in patients with stable diabetes.

In Tables IX and X are presented the clinical findings in the patients who did not fulfill all criteria of the classification. The differences in respect to their classification have been previously discussed. Most of the patients in the first

group have retinopathy, and four have retinitis proliferans. Four patients in this group showed renal impairment and seven presented evidence of vascular damage. In comparison, patients in group II who were classified as possibly having brittle diabetes showed fewer degenerative complications.

COMMENTS

There is a disparity of opinion among physicians regarding treatment of patients with diabetes. Conflicting findings of different workers on the incidence of degenerative complications in controlled or uncontrolled diabetes has further confused this problem. Frequently, patients with long-standing diabetes are seen in whom the blood sugar values have been consistently fluctuating in all extremes in spite of attempts to obtain normoglycemic control and yet who appear to have very slight degenerative complications. Observations of this kind have added further doubts to the importance of normoglycemic control in diabetes mellitus.

It is the authors' belief that these conflicts in opinion and findings result from uncertainties as to definition of the term "control," and from the possibility that error may have been introduced when the occurrence of two types of diabetes mellitus was not accepted. If one assumes that different types of diabetes exist, one is justified in doubting that similar standards of control can be used in both groups.

If one attempts to classify control according to the standards introduced by Wilson, Root and Marble,[15] a number of our patients with brittle diabetes who have been followed consistently for ten to fifteen years and have been giving maximum cooperation would be classified as in excellent or good control in spite of the fact that ideal normoglycemic control never has been obtained. If one attempts to use as criterion the "glycosuric score,"[16] patients with brittle diabetes would be classified as poorly controlled. This criterion is of limited value in patients with brittle diabetes due to the inconsistent correlation between blood sugar values and urinary tests, especially when recorded on single specimens. If one attempts to classify according to the incidence of ketosis, error again is introduced because in persons with stable diabetes complete withdrawal of insulin will produce ketosis only after several days, in contrast to persons with brittle diabetes in whom reduction of the amount of insulin given will produce ketosis in a much shorter time.

In the classification used in this study an attempt has been made, first to separate patients with stable and brittle diabetes and second to express type of control separately in the two groups. Of 172 patients, 149 fulfilled all criteria and were separated into stable and brittle groups. In twenty-three cases such clear separation was not possible. However, when these patients were further classified according to one of the other criteria used, those who showed a high percentage of differences in blood sugar levels exceeding 100 mg. per cent (designated "atypical group II") appeared to have a higher frequency of insulin reaction and ketonuria than the remainder (called "atypical group I") and could be classified as "possibly brittle." We are uncertain how to classify those patients in atypical group I, some of whom never had insulin reactions or never were shown to have ketosis, but did present a V-shaped distribution curve of blood sugar results. (Fig. 1.) It is not clear whether or not this group includes diabetic persons with true insulin sensitivity. Four of twelve patients did present definite signs of renal impairment and uremia and this may have had an influence on their stability. The absence of ketonuria more definitely excludes them from classification as having brittle diabetes.

From this study it is evident that the V-shaped type of distribution curve for blood sugar alone is not an adequate criterion but does suggest that a patient has an unstable type of diabetes. The high per cent incidence of differences in sugar levels of more than 100 mg. per cent seems to be, in conjunction with other criteria, a more reliable index of brittleness.

A further attempt was made to express type of diabetic control. In patients with stable diabetes two types of curves (A and B) were obtained. On theoretic grounds one would expect that patients with type A, who had 60 per cent or more of blood sugars in the lower ranges, would be characterized as good control, and those with less than 60 per cent (type B) as poor control. It was of interest that patients with stable diabetes and with poor control (type B), especially those with long-standing diabetes, had a higher incidence of diabetic retinopathy and of degenerative complications in the cardiovascular system than did patients with stable diabetes with good control (type A).

In patients with brittle diabetes the subdivision into type A and type B was not based on very distinct characteristics. Those who might

be designated in good control differed little from the remainder and the authors were unable to find any difference in the incidence of degenerative complications between these two types.

In comparing the difference in clinical findings in the stable and brittle group, there is a higher proportion of women among cases of brittle diabetes. In comparing the weights of these patients and the differences of ideal and maximum weights, one can conclude that persons who have stable diabetes are more frequently overweight than those who have brittle diabetes. It has been reported by others that the insulin-sensitive type of diabetes is seen mostly in the thin underweight patient. Most patients attained their maximum weights before diabetes had developed. In patients with stable diabetes the weight was reduced from 200 pounds or more to the range of 140 to 160 pounds (average of weight lost, 50 pounds); whereas in patients with brittle diabetes the weight was reduced from the range of 140 to 160 pounds to the range of 120 to 140 pounds (average of weight lost, 20 pounds). This indicates not only that persons with stable diabetes are obese but also that loss or gain of weight in these patients occurs in larger weight ranges. This is in close agreement with the clinical impression that persons with stable diabetes gain weight rapidly if they do not follow their diet strictly, in contrast to those with brittle diabetes who do not gain weight readily.

The studies of Draper, Dupertuis and Caughey,[17] and of Lister, Nash and Ledingham[18] of the constitutional make-up of patients with diabetes indicate that there are two clinical types of diabetes which can be identified by certain typical constitutional stigmas; a close correlation of these types to the presence or absence of insulin sensitivity was shown.

Two other characteristics of persons with brittle diabetes are high incidence of insulin reactions occurring after physical activity and high incidence of insulin lipodystrophy, especially in women. Paley[19] has reported a higher incidence of lipodystrophy in women and in those diabetic persons using protamine zinc insulin. The implications of these differences are not clearly understood. It is the belief of the authors that brittleness cannot be explained on the basis of irregular absorption from the site of insulin lipoatrophy, as has been suggested,[20] because all patients with insulin atrophy consistently avoid these areas for injection; furthermore not all persons with brittle diabetes have lipodystrophy.

The incidence of diabetic retinopathy of grades III and IV was significantly higher in the stable than in the brittle group. The infrequency of grades III and IV retinopathy in persons with brittle diabetes might indicate that there is a lesser degree of severity of complications; however, there is a possibility that retinitis proliferans is not present in this type of diabetes. Ehlers[21] has pointed out that simple and proliferating diabetic retinopathy are two fundamentally different occular disorders and may be caused by two different types of diabetes.

The incidence of cardiovascular damage was much higher in patients with stable diabetes of ten or more years duration, especially in those with poor control. The smaller incidence of arteriosclerosis found in brittle diabetes is not accounted for by the higher ratio of female to male patients seen in this type of diabetes. The work of James, Post and Smith,[22] and others, has indicated a sharp rise in incidence of myocardial infarction in women after the fifth decade. The higher incidence of patients in the age range of sixty-one to seventy years in the group with stable diabetes may account to some extent for the higher incidence of complications in this group. However, it has been shown[23,24] that diabetes mellitus eliminates the relative immunity of women and that of younger patients to atherogenesis.

It is apparent from these findings that if a classification is used in which diabetic subjects are compared only according to control, without taking into consideration types of diabetes mellitus, an error is introduced in evaluating incidence of degenerative complications because, for example, patients with brittle diabetes will be included with those patients having poorly controlled stable diabetes. This possibly may explain the findings of others that poorly controlled diabetic subjects occasionally show no degenerative complications, leaving in doubt the significance of normoglycemic control.

Why adult patients with brittle diabetes have fewer complications is not clear. The possible implication of the adrenal gland as the limiting factor for differences found in the two types of diabetic patients is an intriguing one but no definite substantiating evidence in human beings is available.

Metabolic mechanisms which come into play in fasting patients or in those with impaired carbohydrate utilization, in both of whom ketosis develops, are thought to be quite similar. One

may postulate on this basis that the patient with brittle diabetes in whom ketosis consistently and frequently develops is, metabolically speaking, in a state of repeated "endogenous fasting" in which fat is catabolized much more than it is in the patient with stable diabetes. If this assumption is correct one could possibly explain the differences in weights found in these patients and possibly the smaller incidence of degenerative complications. The mechanism of insulin sensitivity and the liability of these patients with brittle diabetes to the development of ketosis are not as yet clearly understood.

SUMMARY

1. A classification of diabetes, based on the study of 172 patients, is presented. An attempt is made to separate persons with stable diabetes from those with brittle diabetes, and to express diabetic control in these patients.

2. The incidence of degenerative complications was higher in patients with stable diabetes than in those with brittle diabetes.

3. The incidence of severe diabetic retinopathy (grades III and IV) in adult patients with brittle diabetes with long-standing diabetes was very low.

4. Patients with stable diabetes with poor control showed a significantly higher incidence of late degenerative complications than patients with stable diabetes with good control.

5. Further indications are presented to support the concept, already described in the literature, that there are two types of diabetes mellitus which should be looked upon as distinct metabolic entities.

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Observations of Human Adrenal Cortical Deficiency*

With Special Reference to Replacement Therapy with Cortisone

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OVER a period of five years adrenalectomies with or without sympathectomies have been performed in the University Hospital in a large number of patients as an experimental therapeutic approach to severe hypertensive disease. The therapeutic value of the procedures employed is appraised from time to time by a general follow-up study and is not the concern of this report.[1–6] The present report is an account of clinical studies which have been made in a smaller group of these adrenal-deficient patients, together with related data on persons with spontaneous Addison's disease. The results are of interest in respect to some general aspects of the physiology of adrenal-deficient man.

Cortisone and desoxycorticosterone constituted the chief steroid therapy used in these patients over the five-year period. The authors have made a long-term survey of the merits and deficiencies of these agents as substitutes for the secretion of the adrenal cortex. This experience should prove useful as a standard of comparison for future evaluation of newer agents in the management of adrenal deficiency, notably aldosterone and the synthetic halogenated and unsaturated derivatives of hydrocortisone and cortisone.

The studies presented deal particularly with two aspects, namely, (1) whether or not surgically produced primary adrenal deficiency in man differs in kind or in degree from spontaneously occurring Addison's disease, and (2) to what extent cortisone (with or without supplementation with desoxycorticosterone) falls short of furnishing entirely satisfactory maintenance therapy for adrenal-deficient man.

METHODS

This presentation is based in large part upon data collected on a group of patients who, as of March 1, 1955, were being followed weekly in a special adrenalectomy follow-up clinic; those who had been under observation for less than six months following second stage adrenalectomy were excluded from this study. The clinical material is otherwise unselected but it does not include patients who died after adrenalectomy prior to March 1955, nor adrenalectomized private patients not under the care of the follow-up clinic. The duration of follow-up of the adrenalectomized patients reported ranges from six months to five years. All these patients have been seen by one of the authors (A. G. H.) on almost every clinic visit over a four year period. The negative data in the tables are in every case based upon specific questioning and personal examination of individual patients.

Table 1 lists the adrenalectomized patients comprising the material covered in this report. Seventeen patients had been subjected to total bilateral adrenalectomy and twenty-seven to subtotal adrenalectomy, which generally comprised total removal of one adrenal gland and resection of about 95 per cent or more of the other. In one instance (S. W.), however, a remnant of both adrenal glands was left *in situ*. Adrenalectomy was performed in two stages, separated by an interval usually of about ten days, occasionally longer. Follow-up is dated from completion of the second stage procedure. In most of the patients adrenal resection was accompanied on both sides by surgical attack on the sympathetic nervous system, consisting most commonly of excision of the twelfth thoracic and first two lumbar ganglia with interruption of the three splanchnic nerves. The operative details are given elsewhere.[4] Two patients were subjected to a second exploration at the site of the original subtotal adrenal resection with total (patient B. L.) or subtotal removal (patient T. C.) of the residual

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TABLE 1

MINIMAL HORMONE REQUIREMENT AND TWENTY-FOUR HOUR URINARY EXCRETION OF SODIUM AND POTASSIUM ON SELF-SELECTED DIETS

Patient	Sex and Race	Minimal Hormone Requirement*		Melanosis*	Urinary Excretion	
		Oral Cortisone Acetate (mg./day)	Buccal Desoxycorticosterone Acetate (mg./day)		Na (mEq./24 hours)	K (mEq./24 hours)
A. Patients Who Had Undergone Total Adrenalectomy						
A. Be.	F, W	37.5	2	0	.....	.....
P. B.	F, W	37.5	2	0	.....	.....
H. D.	M, W	37.5 † / 37.5	2 † / 0	0	.....	.....
T. D.	M, W	18.8	3	0	.....	.....
S. F.	M, W	37.5	2	0	208; 69	45; 83
F. J.	F, N	25 † / 37.5	2 † / 0	0	.....	.....
B. J.	M, N	50	0	++	169; 334	.....
B. L.	F, W.	37.5	2	0	.....	.....
R. L.	F, W	37.5	1	0	.....	.....
A. Mo.	M, W	25	0	+	135; 70	.....
A. Mu.	F, W	37.5	2	0	.....	.....
F. N.	M, W	37.5 † / 50	2 † / 0	0	87	.....
J. R.	M, W	25	1	0	.....	.....
A. S.	M, W	18.8	2	0	.....	.....
M. S.	F, N	25	2	++	172	44
M. W.	F, W	12.5 † / 25	3 † / 0	0	90; 160	28; 36
J. W.	M, W	25	1	0	.....	.....
	Mean	32.7	1.7			
B. Patients Who Had Undergone Subtotal Adrenalectomy						
I. B.	F, N	0	0	+	.....	.....
A. Bo.	F, W	18.8	1	0	.....	.....
M. C.	F, N	25	1	++	.....	.....
S. C.	F, N	0	0	++	.....	.....
T. C.	M, W	12.5	1	+	.....	.....
H. C.	M, N	6.3	2	++	.....	.....
M. D.	F, N	0	0	++	.....	.....
J. D.	M, W	12.5	1	0	.....	.....
E. D.	M, W	0	0	0	.....	.....
A. F.	F, N	6.3	0	++	.....	.....
B. G.	F, W	6.3	0	0	.....	.....
M. H.	F, W	37.5	0	0	169	58
J. L.	M, W	12.5	0	+++	.....	.....
C. L.	M, W	37.5	1	0	.....	.....
M. M.	F, W	0	0	0	.....	.....
H. M.	F, W	25	1	0	.....	.....
E. M.	M, W	0	0	0	.....	.....
F. M.	M, W	25	0	0	165	62
L. P.	M, W	25	0	0	.....	.....
H. R.	F, W	0	0	0	221	91
M. R.	F, W	6.3	2	++	.....	.....
T. S.	M, W	6.3	2	0	.....	.....
H. T.	F, W	37.5	2	0	.....	.....
J. V.	M, W	37.5	2	0	.....	.....
S. W.	M, N	25	0	+++	.....	.....
F. W.	F, N	18.8	2	++	.....	.....
D. Z.	F, W	0	0	+	.....	.....
	Mean	12.4	0.7			

* See text.

† Minimal requirement for cortisone was determined separately with and without supplementary desoxycorticosterone. The upper figure of the paired values was used in computing the mean values.

adrenal tissue. Follow-up of these patients for purposes of this report dates from the last operation.

Certain studies have also been made of the patients currently under the care of the endocrine clinic in whom Addison's disease had been documented, in order to permit comparison of spontaneous and iatrogenic adrenal deficiency. The patients and diagnostic criteria are listed in Table II.

Determinations on blood or urine were made in a special research laboratory operated exclusively for the study of adrenalectomized patients and the following methods employed: sodium and potassium with the aid of a Baird Associates flame photometer equipped with internal lithium standard, urea by Karr's method,[7] and eosinophils by Manners' method.[8] All twenty-four hour urine collections were checked for accuracy by estimation of twenty-four hour urinary creatinine excretion.[9]* Neutral reducing lipids were measured both in mineral acid (pH 1) hydrolysates and on samples subjected to prior hydrolysis for forty-eight hours at pH 4.5 with β-glucuronidase derived from spleen. Blood glucose concentrations[12] and serum cholesterol concentrations[13] were determined in the William Pepper Laboratory.

OBSERVATIONS AND RESULTS

Determination of Minimal Hormone Requirements. (Table I.) It was a principle in the management of these patients that following adrenalectomy the minimum quantity of adrenal steroids should be administered which was consonant with the patient's continued well-being. Accordingly, the minimal hormone requirement was determined in all of them as follows. Cortisone was administered to all patients in liberal quantity with or, more commonly, without desoxycorticosterone during the early postoperative period after the second stage adrenalectomy. Thereafter gradual stepwise reduction of exogenous steroid therapy was effected until clear-cut clinical and chemical signs of adrenal insufficiency became manifest. Cortisone acetate dosage was reduced in steps of 12.5 mg. per day between the dose levels of 50 and 25 mg. daily, and in steps of 6.25 mg. per day from the dose level of 25 mg. per day to zero. Steroid therapy was then increased for a few days until signs and symptoms of adrenal insufficiency had cleared. Replacement therapy was then provided in the next amount above the dose at which adrenal insufficiency had appeared.

Some months thereafter a second trial on smaller amounts of steroid was made, and in most instances further efforts were made subsequently to reduce the replacement therapy supplied. In several patients the minimum requirement was somewhat elevated during the early postoperative months but by six months after adrenalectomy the hormone requirement had become well stabilized in all patients except S. W. The minimal hormone requirement in this subject was still decreasing two to three years after subtotal adrenalectomy. More detailed data, which suggest that some adrenal cortical regeneration was occurring in this subject, are presented subsequently. The other patients have all been maintained throughout the period of follow-up on the minimal hormone requirements, with the following exceptions: (1) Additional hormone, especially desoxycorticosterone, has generally been given to all patients during the hot summer months as a safeguard against salt depletion due to excess sodium loss in sweat. (2) Additional hormone has been given when necessary for the treatment or prevention of intercurrent episodes of acute adrenal insufficiency. (3) In certain patients extra hormone has been given at times in order to make various experimental observations of the effects of this procedure.

In Tables I and II are shown the minimal hormone requirements, determined as described. The amounts of steroid shown are those which were being administered as of March 1955, except for those given to a few patients who were being subjected at that time to clinical experiments involving manipulation of steroid therapy. A number of the patients were receiving a small quantity (1 to 3 mg.) of buccal desoxycorticosterone acetate in addition to cortisone. This supplementary medication was frequently given to patients having undergone total adrenalectomy, especially those on relatively salt-poor diets by preference, partly because of reported evidence that moderate quantities of cortisone alone may not safeguard the salt balance of adrenal-deficient persons under ordinary circumstances.[14] However, a number of patients having undergone total adrenalectomy have been maintained for many months on 50 mg. or less of oral cortisone without any desoxycorticosterone and, except during the hot summer months, without supplementary salt. Apparently in these patients salt balance was maintained during these periods, because both extracellular fluid volume, as judged by fre-

* The authors are indebted to Dr. F. C. Dohan for determinations of twenty-four hour urinary excretion of total neutral 17-ketosteroids[10] and neutral reducing lipids,[11] which were performed in his laboratory.

TABLE II

PATIENTS WITH SPONTANEOUS ADDISON'S DISEASE

Usual Maintenance Therapy, Diagnostic Observations and Change of Body Weight After Institution of Cortisone Therapy

Patient	Sex and Race	Hormone Administered		Documentation of Diagnosis				Body Weight		
		Oral Cortisone Acetate (mg./day)	Buccal Desoxycorticosterone Acetate (mg./day)	Melanosis*	"A" Value	Acute Adrenal Insufficiency	Eight Hour ACTH Test	Before Cortisone Started (lb.)	One Year† Later (lb.)	Δ (lb.)
D. A.	M, N	0	0	+++	17; 6	+	×	...	....	....
J. C.	M, N	12.5	1	+++	2.5	+	×	157	176‡	+19
G. D.	F, N	25	4	+++	×	+	+	160	182	+22
G. H.	F, N	12.5	0	+++	9.0	+	×	111	133	+22
O. L.	M, N	25	0	0	×	+	+	138	150	+12
D. McG.	M, N	25	2	++	×	0	+	147	176	+28
L. S.	M, N	0§	1‖	+++	×	+	+	...	....	....
T. S.	M, N	12.5	1¶	+++	7	+	×	159	185	+26
H. W.	M, W	25	4	+	4.6	+	+	147	163	+16
	Mean	15.3	1.4							

* See text.

† One-year value interpolated where necessary between two bracketing measurements.

‡ Weight six months after starting cortisone. (No subsequent follow-up.)

§ Occasionally received 12.5 mg. (or less) daily for short periods.

‖ Desoxycorticosterone trimethylacetate 25 mg. every thirty to forty days intramuscularly.

¶ Desoxycorticosterone acetate 225 mg. implanted as pellets subcutaneously at intervals of one year.

quent weighings, and extracellular sodium concentration, as reflected in serum sodium concentration, remained stable throughout. Since approximate salt balance was maintained, the daily dietary sodium intake could be estimated from random sampling of twenty-four hour urine sodium excretion. Values shown in Table I all represent urine samples obtained when no additional salt and no desoxycorticosterone were being given. A range of sodium excretion is thereby defined which corresponds to an intake of 4.0 to 12.0 gm. of sodium chloride per day, and indicates that these patients had selected diets conforming closely to the usual American diet as far as sodium content is concerned.[15]

In order to ascertain what effect addition of a small quantity of buccal desoxycorticosterone acetate to the regimen might have upon the minimal requirement for cortisone, the latter was determined in four patients having undergone total adrenalectomy (H. D., B. J., M. S. and M. W.) on separate occasions with and without desoxycorticosterone. The results are shown in the table as paired values for minimal hormone requirement, both independently determined during the cool months of the year. The results indicate that supplementary buccal desoxycorticosterone acetate in doses of 2 to 3 mg. exerts very little influence upon the minimal requirement for cortisone under the conditions of the determination, notably self-selection of diet. It might well be, of course, that the cortisone-sparing activity of desoxycorticosterone would be quite appreciable if the minimal hormone requirement was determined while dietary sodium chloride was drastically restricted. Most of the patients have at some time been maintained for a trial period on cortisone alone. In most instances provision of desoxycorticosterone supplements is to be regarded as a safeguard against diminished salt intake and against excessive sodium loss in sweat in the summer, rather than as an actual need of the patient who has undergone total adrenalectomy and been treated with cortisone.

Two patients have been encountered, however, in whom desoxycorticosterone appeared to be required if no more than moderate amounts of cortisone were given. In these patients (S. W. and F. W.) desoxycorticosterone was not needed

to maintain salt balance but was needed because, when it was not administered, blood urea and serum potassium concentrations rose to abnormally high levels (Figs. 1 and 2) despite provision of up to 50 mg. of cortisone acetate orally daily. These manifestations occurred in the absence of the familar accompanying signs of adrenal insufficiency such as postural hypotension, anorexia and weakness. Thorn et al.[16] have mentioned similar observations in a patient having undergone adrenalectomy for hypertensive disease. The syndrome can perhaps most plausibly be ascribed to diminished responsiveness to adrenal hormones of kidneys already damaged, possibly in some selective manner, by vascular disease. In any case, these patients illustrate a distinctly unusual circumstance which may be encountered in the adrenal-deficient patient, namely that a small quantity of supplementary desoxycorticosterone appears to permit the maintenance dose of cortisone to be kept within reasonable limits.

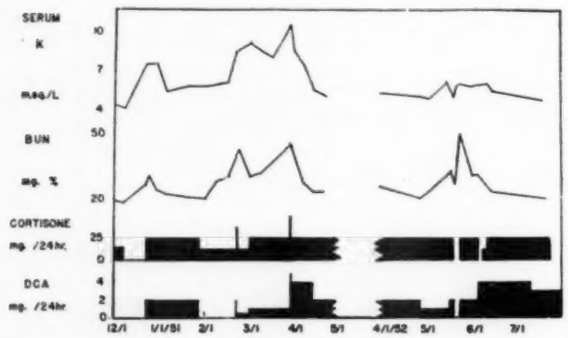

FIG. 1. Patient S. W., Negro man aged 48, subtotal adrenalectomy. The chart illustrates parallel elevation of serum potassium and urea as a manifestation of adrenal insufficiency, and effect of cortisone (oral cortisone acetate) and DCA (buccal desoxycorticosterone acetate).

Measurement of Twenty-four Hour Urinary Excretion of Steroids and Estimation of Adrenal Cortical Reserve. Table III compares the results of twenty-four hour corticotrophin tests conducted on adrenal-deficient patients with comparable data obtained in six normal volunteer subjects and reported elsewhere in detail.[17] The twenty-four hour urinary steroid excretion was measured before corticotrophin was administered and again over a twenty-four hour period beginning with the eight hours over which corticotrophin was infused. Eosinophil counts were made at the beginning and end of the infusion. Except as otherwise noted in the table, control and ACTH values each represent a single determination. Response to ACTH in the two patients having undergone total adrenalectomies were studied with patients taking the usual oral replacement therapy on both days of the test, but during both control and ACTH testing and for at least twenty-four hours before the control day oral steroid was withheld from patients having undergone subtotal adrenalectomies. None of these patients had received any steroid intramuscularly for four weeks or more.

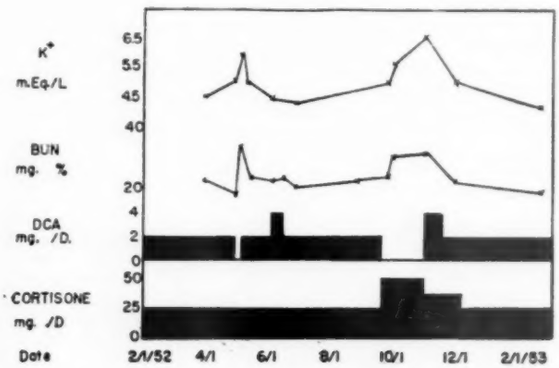

FIG. 2. Patient F. W., Negro woman aged 28, subtotal adrenalectomy. See Figure 1 for explanation.

It is evident from the data presented in the table that the estimation of twenty-four hour urinary steroid excretion alone is of little value in the diagnosis of adrenal deficiency. Adrenal-deficient persons commonly show low values, but values for 17-ketosteroids within the normal range for this laboratory (above 6 mg. per twenty-four hours for women or 8 mg. per twenty-four hours for men) were found in four (H. L., M. M., B. L. and E. M.) of seventeen such patients (24 per cent), and normal values (10 mg. per twenty-four hours or more) for neutral reducing lipids after hydrolysis with glucuronidase were still more frequently encountered. When it is recalled that low values for urinary steroid excretion are frequently encountered in clinical conditions unassociated with gross adrenal disease,[18] the inadvisability of placing much reliance upon twenty-four hour urinary steroid excretion for the diagnosis of adrenal deficiency will be manifest.

Of the three types of steroid estimation which were made, only two are sensitive indicators of adrenal stimulation by ACTH as judged by the increment of the experimental sample over that of the control sample of normal volunteer sub-

TABLE III

RESPONSES OF ADRENAL-DEFICIENT SUBJECTS TO EIGHT HOUR INTRAVENOUS INFUSIONS OF CORTICOTROPHIN (10 UNITS) COMPARED WITH THAT OF NORMAL VOLUNTEER SUBJECTS*

Patient	Status	Steroid Replacement Required	Eosinophil Count†			Urinary Neutral Reducing Lipids†						Urinary 17-Ketosteroids†		
						pH 1			Glucuronidase					
			Per mm.³	Δ	Δ %	mg./24 hr.	Δ mg.	Δ %	mg./24 hr.	Δ mg.	Δ %	mg./24 hr.	Δ mg.	Δ %
M. S.	Total adrenalectomy	+	213	.....	.....	4.9	.....	.....	18.4	.....	.....	2.4	.....	.....
			84	−129	−60	7.1	+2.2	+45	20.2	+1.8	+10	3.1	+0.7	+30
M. W.	Total adrenalectomy	+	321	.....	.....	×	.....	.....	11.8	.....	.....	2.4	.....	.....
			257	−64	−20	×	×	.....	14.7	+2.9	+25	2.5	+0.1	+4
		Mean	...	−96.5	−40	....	+2.2	+45	.....	+2.35	+17.5	.....	+0.4	+17
H. L.	Subtotal adrenalectomy	+	419	.....	.....	2.0	.....	.....	5.5	.....	.....	(10.2‡)	.....	.....
			231	−188	−45	1.2	−0.8	−40	3.3	−2.2	−40	(2.9‡)	(−7.3)	(−72)
T. S.	Subtotal adrenalectomy	+	153	.....	.....	....	.....	.....	.....	.....	.....	.....	.....	.....
			51	−102	−67	2.4	.....	.....	6.2	.....	.....	6.0	.....	.....
S. W.	Subtotal adrenalectomy	+	213	.....	.....	2.1	−0.3	−13	7.5	+1.3	+20	6.5	+0.5	+7
		+	76	−137	−64	2.3	.....	.....	3.6	.....	.....	2.5	.....	.....
J. C.	Subtotal adrenalectomy	+	138	.....	.....	1.1	−1.2	−52	3.2	−0.4	−11	2.7	+0.2	+8
			103	−35	−25	1.4	.....	.....	×	.....	.....	1.8	.....	.....
J. L.‖	Subtotal adrenalectomy	+	287	.....	.....	1.9	+0.5	+36	×	×	×	1.5	−0.3	−17
			243	−44	−15	×	.....	.....	14.2	.....	.....	4.9	.....	.....
						×	×	×	5.2	−9.0	−64	4.6	−0.3	−6
		Mean	...	.....	−43	....	−0.45	−17.25	.....	−2.58	−24	.....	−0.02	−2
B. G.	Subtotal adrenalectomy	0	320	.....	.....	2.8	.....	.....	×	.....	.....	5.5	.....	.....
			205	−115	−36	3.0	+0.2	+7	×	×	×	6.0	+0.5	+9
M. M.	Subtotal adrenalectomy	0	138	.....	.....	2.3	.....	.....	9.2	.....	.....	8.4	.....	.....
			125	−12	−9	2.5	+0.2	+8	8.9	−0.3	−3	8.4	0	0
B. L.	Subtotal adrenalectomy	0	259	.....	.....	3.4§	.....	.....	8.7§	.....	.....	7.4§	.....	.....
			34	−225	−87	3.4	0	0	12.0	+3.3	+38	6.2	−1.2	−16
E. M.	Subtotal adrenalectomy	0	100	.....	.....	3.1§	.....	.....	21.4§	.....	.....	14.7§	.....	.....
			69	−31	−31	4.1	+1.0	+32	22.4	+1.0	+5	15.8	+1.1	+7
I. B.	Subtotal adrenalectomy	0	494	.....	.....	×	.....	.....	9.9	.....	.....	4.9	.....	.....
			457	−37	−7	×	×	×	12.6	+2.7	+27	3.6	−1.3	−26
E. D.	Subtotal adrenalectomy	0	115	.....	.....	4.3	.....	.....	9.4	.....	.....	6.6	.....	.....
			59	−56	−49	3.7	−0.6	−14	13.7	+4.3	+46	7.3	+0.7	+11
		Mean	...	.....	−36.5	....	+0.16	+6.6	.....	+2.2	+26	.....	−0.04	−2.5
M. J.	Addison's disease	0	218	.....	.....	×	.....	.....	×	.....	.....	2.5	.....	.....
			280	+62	+28	×	×	×	×	×	×	2.0	−0.5	−20
H. W.	Addison's disease	+	156	.....	.....	2.1	.....	.....	10.5	.....	.....	7.6	.....	.....
			123	−33	−21	3.5	+1.4	×	7.3	−3.2	−30	7.5	−0.1	−1
O. L.	Addison's disease	+	119	.....	.....	×	.....	.....	15.3	.....	.....	4.2	.....	.....
			73	−46	−39	×	×	×	11.6	−3.7	−24	4.3	−0.1	+2
D. McG.	Addison's disease	+	382	.....	.....	×	.....	.....	×	.....	.....	3.1	.....	.....
			225	−157	−41	×	×	×	×	×	×	2.7	−0.4	−13
		Mean	...	.....	−18	....	.....	.....	.....	−3.45	−27	.....	−0.22	−8
Mean of 5 normal volunteer subjects¶	.....	0	...	.....	−96	....	+2.6	+57	.....	+12.1	+102	.....	+10.0	+77
			...	.....	±2.4	....	±1 55	.....	.....	±3.1	.....	.....	±4.71	.....

* See text.
† Experimental values below the control values.
‡ Steroid administered on day of control, not on day ACTH was given; Δ omitted from mean.
§ Mean of two control determinations.
‖ Steroid replacement provided both on day of control and on day of ACTH administration.
¶ Values for five normal volunteer subjects based on data presented elsewhere;[17] 95 per cent confidence limits shown.

jects. Neutral reducing lipids determined after hydrolysis with mineral acid at pH 1 showed a rise of only 2.6 mg. per twenty-four hours in the volunteer subjects. This increment is so small in comparison with the control values and the random variation is so great that the use of this determination as an index of adrenal responsiveness cannot be recommended.

TABLE IV
INCIDENCE OF MELANOSIS FOLLOWING ADRENALECTOMY*

Race	Type of Adrenalectomy	Number of Patients	Number Pigmented	Incidence of Melanosis (%)
Caucasians	Total	14	0	0
	Subtotal	19	4	21
Negroes	Total	3	3	100
	Subtotal	8	8	100

* Data derived from Table I.

On the other hand, both neutral reducing lipids, as determined following β-glucuronidase hydrolysis of the urine, and 17-ketosteroids are very useful determinations for the purpose. Large increments in the twenty-four hour urinary excretion of these substances (mean +12.1 and +10.0 mg. per twenty-four hours, respectively) were regularly observed following corticotrophin administration in normal volunteer subjects but were not observed in the adrenal-deficient subjects. The mean difference between the responses of normal volunteer subjects and those of adrenal deficient subjects in this respect is highly significant. In no instance did an adrenal-deficient subject show an increment of urinary excretion of 17-ketosteroids or neutral reducing lipids (determined on glucuronidase hydrolysates) approaching the smallest increment observed in the volunteer subjects.

The number of eosinophils per cubic millimeter of blood declined in all but one adrenal-deficient person following corticotrophin administration. The fact that the eosinophil count regularly decreased in patients having undergone subtotal adrenalectomy who had received no oral hormone for twenty-four hours or more indicates that corticotrophin could induce a small increase of secretion by residual adrenal cortical tissue in these adrenal-deficient persons. Minute quantities of adrenal hormone undoubtedly affect the number of circulating eosinophils, but it seems clear[17] that sizable non-specific variations of the eosinophil count occur; therefore a decrease of as much as 40 per cent may not necessarily signify that any increased secretion of adrenal hormone has taken place. Even a decrease of 65 per cent of circulating eosinophils, although presumably implying that some slight adrenal stimulation has been accomplished, obviously does not establish the presence of adrenal reserve of any clinical significance, since decreases of this magnitude may occur in persons who are severely adrenal-deficient by all other criteria. However, with a single exception, no adrenal-deficient patient showed a decrease of circulating eosinophils exceeding 67 per cent.

Normal volunteer subjects, in contrast, all responded to corticotrophin infusion with almost complete disappearance of circulating eosinophils (mean change = −96 per cent); in none was there a decrease of as little as 90 per cent.[17] When a series of miscellaneous hospitalized patients (excluding those with gross adrenal disease) was similarly studied, decreases of as little as 84 per cent were occasionally encountered.[19,20] Decreases less than this are not seen in the absence of gross adrenal deficiency, provided of course that the testing is technically valid. Circumstances which may invalidate the use of the eosinophil index are discussed elsewhere.[19,20]

It is clear that none of the adrenalectomized patients studied showed any consequential response to intravenously administered corticotrophin in terms of urinary steroid excretion, while in all but one instance the response in terms of change of the eosinophil count was grossly subnormal. The one patient (B. L.) who responded with a decrease of 87 per cent circulating eosinophils was able to live a normal life without receiving any exogenous hormone and was subsequently found to harbor a sizable cortical remnant.[17] It may therefore be inferred that a normal decrease of circulating eosinophils in response to the standard corticotrophin infusion, although incompatible with severe adrenal deficiency, does not exclude the possibility of markedly reduced cortical reserve.

Addisonian Melanosis in Adrenalectomized Man. (Tables I, II and IV.) Melanosis indistinguishable from the pigmentation of Addison's disease has developed in a number of these adrenalectomized patients. Melanosis appeared, if at all, within three months after the second stage but sometimes became more noticeable after many

months in the form of sun tan which persisted after the summer season. The distribution and type of darkening resembled practically all varieties encountered in Addison's disease, including increasing tendency to tan in sunlight, diffuse darkening of the entire skin, localized darkening of the palmar creases, extensor surfaces of the elbows and exposed areas, pigmentation of recent scars, darkening of the areolae, and appearance of pigmented maculae on skin and mucous membranes. Vitiligo has not been seen. The rough grading of the intensity of the pigmentation shown in Table I–III was made on the basis of simple inspection. Because the authors have never seen convincing evidence of melanosis in persons with Addison's disease that did not include darkening of the palmar creases, whether *de novo* or in the form of increase of preexisting constitutional pigmentation, this sign has been regarded as certain evidence of the minimal melanosis of Addison's disease. When it has been accompanied by any definite localized pigmentation elsewhere the grade ++ has been assigned, the grade +++ being reserved for intense disseminated melanosis. Although the occurrence of severe Addison's disease without pigmentation is beyond question, melanosis is said to be present in 90 per cent or more of patients with Addison's disease.[21–23] Pigmentation was present in all but one of the nine patients in the present series who had spontaneous Addison's disease and in seven of them it was marked. (Table II.) By contrast, definite melanosis developed in but twelve of twenty-seven patients having undergone subtotal adrenalectomy (44 per cent) and in only three of seventeen patients having undergone total adrenalectomy (18 per cent).

Taken at face value, the data in Tables I and II would seem to indicate (1) that the incidence of melanosis in adrenal deficiency is markedly and significantly lower after adrenalectomy than when the result of morbid processes, and (2) that the incidence of melanosis in adrenalectomized man is markedly and significantly lower when both adrenal glands are removed *in toto* than when a small remnant is spared.

The second inference, however, is largely or entirely illusory, as is evident when the data are partitioned on the basis of race. (Table IV). It is apparent that in this series in the grossly adrenal-deficient Negro the melanosis of Addison's disease almost invariably developed, irrespective of the manner in which deprivation of adrenal tissue took place. Among the Caucasians an apparent difference remains between total and subtotal adrenalectomy with respect to the incidence of pigmentation but the difference is not significant by the chi-square test. Obviously it would be misleading to compare different series of adrenal-deficient patients for the purpose of relating the incidence of melanosis to the cause of adrenal deficiency unless suitable correction was made for the racial composition of the series. Indeed it may be supposed that even within the Caucasian group the racial stock or the complexion of the patients may affect the incidence of the melanosis of adrenal deficiency, for it is said that the darkening is usually less conspicuous in blondes.[22]

Thus there is an undeniably low incidence of melanosis in persons with Addison's disease who have been rendered adrenal-deficient by surgery (as has also been noted by others[16,24–28]) compared with the high reported incidence in persons with spontaneous Addison's disease. Nevertheless, a fundamental distinction in this respect between spontaneous and surgically induced adrenal deficiency cannot be made because almost all adrenalectomized persons, unlike patients with Addison's disease, have been maintained on exogenous cortisone from the inception of adrenal deficiency; cortisone has been shown to be capable of causing some regression of the melanosis of Addison's disease.[25] Furthermore, pronounced melanosis was reported to have developed[29] in one patient to whom no cortisone was administered during or after adrenalectomy, and pigmentation not previously present in two adrenalectomized patients maintained on cortisone appeared within two weeks after withdrawal of the cortisone.[28] Finally, although discussion of the etiology of the melanosis of Addison's disease[25,30,31] is beyond the scope of this article, it may be taken for granted that the pigmentation is, in the final analysis, a consequence, mediate or immediate, of adrenal cortical deficiency. Clinico-pathologic evidence[32] strongly contravenes the notion that the adrenal medulla plays any role in the production of this or any other manifestation of Addison's disease. It is, therefore, difficult to conceive that the manner in which deprivation of adrenal cortical tissue takes place could possibly constitute *per se* a factor capable of affecting the incidence of the ensuing melanosis.

One may grant that cortisone exerts antipigmentary effects in adrenal-deficient subjects and

that cortisone therapy can account for the relatively low incidence of pigmentation in those cases of adrenal deficiency which are due to adrenalectomy without implicating cortisone and hydrocortisone as the major or sole constituents of the adrenal secretion responsible for

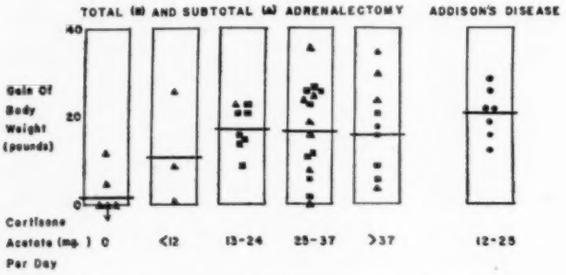

FIG. 3. Gain of body weight in one year in relation to cortisone therapy in adrenal-deficient subjects.

preventing the melanosis of Addison's disease in normal persons. The following evidence suggests that another adrenal hormone (or hormones) is more important in the physiologic control of melanosis.

(1) Although it is widely believed that cortisone and hydrocortisone frequently diminish melanosis in patients with Addison's disease, a clinical impression gained also in several of the authors' own patients, and although there is objective evidence that slight depigmentation can be produced in adrenal-deficient subjects with melanosis by cortisone,[25] no evidence has been reported that complete regression of the abnormal pigmentation occurs regularly, if at all, as a result of prolonged therapy with small amounts of cortisone or hydrocortisone over periods as long as seven years. No clinically detectable depigmentation has been observed in this clinic as a result of short-term experimental administration of large quantities of cortisone (up to 100 mg. of the acetate orally daily for twenty-one days) to deeply pigmented adrenal-deficient subjects. It could be argued that melanosis, once established, may be to some extent irreversible, but certain evidence, to be presented, may be contrary to this contention.

(2) Observations, to be described, of one patient having undergone subtotal adrenalectomy suggest that regeneration of adrenal cortical tissue occurred and was associated with marked regression of melanosis. This would indicate the presence in the adrenal secretion of a factor capable, as maintenance therapy with cortisone or hydrocortisone apparently is usually not, of bringing about striking regression of the pigmentation.

(3) There is reason to suppose that adrenal-deficient patients receiving their minimal hormone requirement largely or wholly in the form of cortisone actually secure more 11-17-oxygenated alpha ketolic steroid thereby than is provided in the usual secretion of normal glands. Since in many adrenal-deficient patients so treated pigmentation nevertheless occurs, it would follow that some other component of the adrenal secretion is partly responsible for the failure of normal persons to develop the melanosis of Addison's disease.

Gain of Body Weight in Relation to Cortisone Therapy. (Fig. 3.) Certainly the most common and possibly the most troublesome problem encountered in caring for these adrenal-deficient patients who are being maintained on cortisone therapy has been excessive appetite and resultant weight gain. Prescription of low calorie diets, exhortations to restrict food intake and administration of anorexigenic drugs have helped to control weight but have by no means obviated the problem. No studies of body composition were undertaken in these patients but it was generally evident from simple inspection that weight gains reflected chiefly accumulation of adipose tissue.

In Figure 3 are plotted the gains of body weight observed in adrenal-deficient persons over one year after cortisone administration was begun and are presented in relation to the average amount of cortisone administered. In the case of patients with Addison's disease the weight gains shown are measured for one year from the last available record of weight before cortisone therapy was started, which was in all but one instance on the same day steroid administration was begun. Of the seven patients treated with cortisone who had Addison's disease four (J. C., G. H., D. McG. and T. S.) had been maintained on desoxycorticosterone acetate for months to years prior to the exhibition of cortisone, while three (G. D., O. L. and H. W.) were all treated with cortisone as soon as the diagnosis was made.

In patients having undergone adrenalectomy weight gains are calculated over a period of twelve months beginning two months after completion of the final operation. By this time the preoperative weight had usually been reattained and the minimum hormone requirement either ascertained or approached. All adrenalecto-

mized patients listed in Table 1 are represented in the figure with the following exceptions: S. F. was omitted because of the complication of overt diabetes mellitus first detected eight months after operation; B. L. was omitted because she had been pregnant for ten weeks at the time total adrenalectomy was performed; and P. B. and J. L., for whom records of body weight are inadequate for present purposes, were also omitted.

The data represented in Figure 3 indicate that the average rate of gain of body weight during the first year of cortisone administration to adrenal-deficient persons increases as the average maintenance dose is increased to a limiting value of about 25 mg. of cortisone acetate per day. In general the duration of marked gain in weight has usually been about one year, sometimes somewhat longer. Several patients, however, continued to exhibit marked weight gain for as long as three years.

It is noticeable in Figure 3 that the mean rate of weight gain of the patients with Addison's disease was greater than that of the patients having undergone adrenalectomy maintained on similar amounts of cortisone. The difference may be attributable to the fact that the baseline in the case of the patients having undergone adrenalectomy was a state of eucorticism, whereas the patients with Addison's disease started at a weight which reflected prior insufficiency of adrenal secretions, including the 11-17-oxygenated alpha ketolic steroids.

The marked stimulation of the appetite of patients with Addison's disease produced by replacement therapy with cortisone has been noted by others.[14] A striking feature of the increased appetite and weight gain of these adrenalectomized patients is that the body weights attained fourteen months after initiation of cortisone therapy were in almost all instances well beyond the usual weights of these patients at a time when the adrenal gland was functioning normally, and that after several years on minimal maintenance therapy a number of these patients have become extremely obese. Clearly the effect of these relatively small doses of cortisone upon the appetite is excessive and is similar to the effect produced by large doses of cortisone in patients with various diseases not ascribable to abnormal function of the adrenal gland. It must be concluded that these adrenal-deficient patients, in respect to stimulation of appetite and weight gain, have exhibited evidence of hypercortisonism as a result of receiving the minimal hormone requirement largely or wholly in the form of cortisone.

Glucose Tolerance and Responsiveness to Hypoglycemia. (Table v.) Seventeen patients having undergone adrenalectomy were subjected to a standardized oral glucose tolerance test. The blood sugar was determined after an overnight fast, then 1.0 gm. of glucose was administered orally per kilogram of ideal body weight over a fifteen minute period. The blood sugar was determined at intervals after the completion of glucose ingestion as shown in Table v. Those patients maintained on exogenous steroid therapy continued to take medication before and after the test; the usual morning dose of oral cortisone acetate (12.5 mg. or less) was given immediately before the start of the test.

Abnormalities of two kinds are observable in the table, impaired glucose tolerance and late, often persistent, hypoglycemia. There is general agreement[33] that the results of the standard glucose tolerance test are considered abnormal if the blood sugar (determined by the Somogyi method) exceeds 170 mg. per 100 ml. one hour after glucose ingestion, 120 mg. per 100 ml. two hours thereafter, or 110 mg. per 100 ml. three hours thereafter. According to these criteria, three (S. F., B. J. and S. W.) of fifteen patients having undergone adrenalectomy who were maintained on cortisone therapy exhibited evidence of impaired glucose tolerance (20 per cent). The + signs shown in the table indicate the number of abnormally elevated values (surrounded by circles) which were obtained in each test. Two of these three patients showed a marked "diabetic" type of tolerance curve. One (patient S. W.) has never as yet shown spontaneous glycosuria or any symptoms or signs of diabetes mellitus. In the other (S. F.) frank diabetes mellitus with glycosuria developed and weight loss was noted eight months after adrenalectomy and five months after the glucose tolerance test shown in the table.

Most of the glucose tolerance tests recorded in the table reflect an entirely different abnormality, one highly characteristic of adrenal cortical inadequacy. Whereas normal subjects given the oral glucose tolerance test have blood sugar concentrations two and three hours after glucose ingestion which are, on the average, equal to the fasting value, patients with Addison's disease not only have blood glucose concentrations well below the fasting levels three

TABLE V

STANDARD GLUCOSE TOLERANCE TESTS OF ADRENALECTOMIZED PATIENTS

Patient	Type of Adrenalectomy	Steroid Replacement Therapy	Blood Glucose Concentration* (mg. per 100 ml.)						Symptoms	Impaired Glucose Tolerance	Unresponsiveness to Hypoglycemia
			Fasting	Hours after Glucose Ingestion							
				½	1	2	3	4			
A. B.	Total	+	70	107	76	73	85	77	None	0	0
H. D.	Total	+	73	130	74	91	**42**	**54**	Shaky, sweaty at 3 to 4 hours	0	+
T. D.	Total	+	73	120	65	75	**50**	**64**	None	0	+
S. F.	Total	+	63	145	167	*179*	*167*	92	None	++	?
F. J.	Total	+	84	96	113	112	**82**	**70**	None	0	+
B. J.	Total	+	78	117	159	*139*	96	**52**	Shaky, sweaty at 3 to 4 hours	+	+
R. L.	Total	+	78	107	84	67	**60**	**60**	None	0	+
A. M.	Total	+	70	130	114	87	86	80	None	0	0
F. N.	Total	+	75	89	75	83	75	**65**	None	0	+
E. S.	Total	+	73	110	87	70	**69**	76	None	0	+
J. W.	Total	+	66	109	129	82	**54**	**50**	None	0	+
J. D.	Subtotal	+	79	124	119	68	**68**	**77**	None	0	+
M. R.	Subtotal	+	66	167	160	73	70	70	None	0	0
T. S.	Subtotal	+	59	72	55	61	**44**	63	Giddy, sweaty at 3 to 4 hours	0	+
S. W.	Subtotal	+	122	177	*183*	*162*	*162*	112	None	+++	?
E. D.	Subtotal	0	73	137	143	*130*	92	**44**	None	+	+
E. M.	Subtotal	0	65	113	100	66	**47**	**42**	Weak, sweaty at 3½ to 4 hours	0	+

* Values three and four hours after glucose ingestion which are lower than the fasting value are in bold type. Italicized numbers are one, two, and three hour values which are considered abnormally high. See text.

hours after glucose ingestion but they may remain hypoglycemic for a prolonged period unless treated.[34] The feature characteristic of adrenal cortical inadequacy is not so much that the falling sugar concentration overshoots and falls below the fasting level, but rather that the incipient hypoglycemia does not at once call forth, as it does in the normal person, the physiologic response restoring blood sugar concentration to normal. For this reason Fraser et al.[35] have characterized the essential defect demonstrable in the insulin tolerance test, as well as that in the four-hour glucose tolerance test, as "unresponsiveness to hypoglycemia."

Twelve patients listed in Table v exhibited blood sugar concentrations below the fasting values either three or four hours or both after glucose ingestion. In four of these patients signs and symptoms of hypoglycemia developed. The lowest blood sugar concentrations were recorded in these patients, a level of 52 mg. per 100 ml. or lower having been demonstrated in all four. There can be no question in these four instances that the data establish pathologic unresponsiveness to hypoglycemia. Individually considered, the tolerance curves of certain other patients (for example, F. J. or F. N.), in whom the three or four hour blood glucose concentrations were not markedly below the fasting values, could perhaps not with assurance be stigmatized as abnormal. But it is clear that unresponsiveness to hypoglycemia is highly characteristic of the patients as a group. Since in two patients, S. F. and S. W., glucose tolerance was impaired to a degree sufficient to prevent return of blood sugar concentration to values approaching the fasting values within four hours, the tests could not disclose whether

or not unresponsiveness to hypoglycemia was or was not present. Of the remaining thirteen patients receiving cortisone, only three (A. B., A. M. and M. B.) failed to exhibit three or four hour values below the fasting blood sugar concentration. The data may be summarized as disclosing presumptive or conclusive evidence of unresponsiveness to hypoglycemia in ten of thirteen patients receiving cortisone (77 per cent), as well as in both the patients having undergone subtotal adrenalectomy who did not require steroid maintenance therapy. In this respect there is no indication of any difference between subtotally and totally adrenalectomized persons maintained on steroid replacement therapy.

Certain patients, especially F. N. and T. S., showed a distinctly flat oral glucose tolerance curve, which is a characteristic although not an invariably demonstrable manifestation of adrenal deficiency and which has been shown[34] to reflect delayed absorption of glucose by the gastrointestinal tract. It is evident that some of these adrenal-deficient patients were able to absorb glucose quickly enough to exhibit abnormally pronounced hyperglycemia following glucose ingestion, but it is possible that delayed glycose absorption could in some instances (for example, patient F. J.) have masked impairment of glucose tolerance.

Impairment of glucose tolerance in patients having undergone total adrenalectomy who were maintained on cortisone has been noted by some[26] but not by all[28] observers. In these other series the amount of cortisone used for replacement therapy has been somewhat greater than the minimal replacement therapy given to the authors' totally adrenalectomized patients. No doubt it is for this reason that hypoglycemia and abnormalities of the glucose tolerance curve characteristic of adrenal cortical deficiency have not been noted in these other series.[28] In the authors' patients the glucose tolerance tests have clearly exhibited features of both hypoadrenalism and hypercortisonism, sometimes in one and the same test. Is the seeming incongruity of these two aspects of the tests a reflection of an unusual and characteristic physiologic state in the cortisone treated, adrenal-deficient patient, or can it be accounted for by the timing of the tests in relation to cortisone medication?

The unresponsiveness to hypoglycemia cannot plausibly be ascribed to the timing of cortisone therapy. The maximum activity of orally administered cortisone is achieved about four hours after its administration.[36] Since the morning medication was taken shortly before the test was started the patients must have been, at the time hypoglycemia developed, as much under the influence of administered cortisone as at any time during the day or night on the usual therapeutic regimen. It follows that some hypoadrenalism, as manifested by this criterion, is present at all times in these patients.

By the same token it cannot be denied that the immediate effect of the cortisone taken before the test may have contributed to the impairment of glucose tolerance. Yet it is very difficult to believe that the direct, immediate effect of the quantity of cortisone acetate taken (12.5 mg. or less) could account for such marked abnormal rises of blood sugar as are shown in four instances in the table, since persons without adrenal disease can often tolerate several hundred times this quantity of cortisone daily without glycosuria developing. It seems necessary to postulate that impaired glucose tolerance reflects rather a metabolic steady state resulting from prolonged medication with cortisone and possibly involving changes in the functioning of other organs concerned with carbohydrate metabolism, such as the liver, pancreas or hypophysis. It is true that one patient (E. D.) not requiring cortisone shows a tolerance curve which suggests similar diabetic diathesis but this patient too had been given cortisone for some months following adrenalectomy. It may also be recalled that prolonged corticotrophin stimulation appears to increase the proportion of 11-17-oxygenated steroids in the adrenal secretion of rabbits.[37] If the residual adrenal tissue is being stimulated maximally by endogenous corticotrophin, patients having undergone subtotal adrenalectomy who do not require exogenous hormone may have a higher proportion of cortisone-like steroids in their adrenal secretion than do normal persons.

In this connection it may be mentioned that frank diabetes mellitus, which appeared after adrenalectomy in patient S. F., developed also in a cortisone treated adrenalectomized patient, included in the larger series of patients,[1–6] upon whom surgery was performed. The incidence of diabetes mellitus at the end of five years in the entire series of patients is two in 110 living patients, a figure much above the expected

TABLE VI

WATER TESTS OF TOTALLY ADRENALECTOMIZED PATIENTS*

Patient	Volume of Urine		Urine Urea Nitrogen (mg./100 ml.)	Urine Chloride (mEq./L.)	Serum Urea Nitrogen (mg./100 ml.)	Serum Chloride (mEq./L.)	"A"
	Overnight (ml.)	Largest A.M. Hour Volume (ml.)					
T. D.	635	245	772	53	20	103	17.8
F. J.	285	128	666	165	16	99	11.2
A. Mu.	660	94	47	113	32	113	0.2
F. N.	360	240	84	102	20	106	3.3
M. S.	820	230	600	137	16	102	7.8

* See text.

incidence of new cases in the general population.* This fact lends further support to the view that cortisone administered for a prolonged period, even in the modest quantities commonly used for adrenal replacement therapy, may have diabetogenic properties in susceptible subjects.

Clinical hypoglycemia, although uncommon in these adrenalectomized patients, has occurred. Mild hypoglycemic episodes may of course be overlooked, and are in any case difficult to document, but at least three patients having undergone total adrenalectomy (H. D., S. F. and B. J.) experienced for a time a series of repeated and unmistakable hypoglycemic attacks. It is pertinent that these attacks yielded not to increased frequency and amount of cortisone medication but to spacing of the feedings and attention to the relation of physical exercise to food intake.

Water Tests. (Table VI.) The test procedure was essentially that of Levy, Power and Kepler.[39] Urine volume was collected from 10:30 P.M. to 7:30 A.M. The largest urine volume of four hourly morning collections is shown (8:30 A.M. to 12:30 P.M.), water in an amount equal to 20 ml. per kilogram of body weight having been administered at 8:30 A.M. Urine chloride and urea concentrations in the overnight urine are shown; the values for serum chloride and serum urea nitrogen were measured in the morning. The "A value" was calculated as follows:

$$A = \frac{\text{Largest A.M. urine volume}}{\text{Night urine volume}} \times \frac{\text{Urine urea N (mg./100 ml.)}}{\text{Serum urea N (mg./100 ml.)}} \times \frac{\text{Serum Cl (mEq./L.)}}{\text{Urine Cl (mEq./L.)}}$$

In all patients tested more urine was excreted during the overnight period than during any hourly period following water ingestion and in all patients values less than 25 for A were abnormal, some being extremely low. Although cortisone in sufficient dosage can correct or mitigate the impairment of the diuretic response to water intake characteristic of Addison's disease, the quantities used for maintenance in our patients will apparently not do so. This has been noted also in patients with spontaneous Addison's disease who have been given similar amounts of cortisone.[14]

Effect of Subtotal Adrenalectomy and Minimal Hormone Therapy upon Serum Cholesterol Concentration. (Table VII.) Total serum cholesterol concentration was measured before and at intervals after subtotal adrenalectomy in eight patients, three of whom required some exogenous

* In the population sample covered by the National Health Survey of 1935 to 1936,[38] there were 4.0 more diabetic men and 2.5 more diabetic women per 1,000 persons aged forty-five to fifty-four than in the thirty-five to forty-four age group. Roughly one may estimate from these figures that about 3.7 new cases of diabetes mellitus will appear over any ten year period per 1,000 persons who are age forty-four at the beginning of that period. Since the incidence of new cases of diabetes mellitus is higher in this age group than at any lesser age, and since practically all our patients were younger than fifty when adrenalectomy was performed, it follows that the incidence in the authors' series of one new case of diabetes in forty-four persons, or two cases in 110 persons, followed for a maximum period of five years is far in excess of the expected figure.

TABLE VII
SERUM CHOLESTEROL CONCENTRATION BEFORE AND AFTER SUBTOTAL ADRENALECTOMY

Patient	Serum Cholesterol (mg./100 ml.)		
	Preoperative	Postoperative	
		0–2 yr.	2–5 yr.
A. Not Requiring Exogenous Steroid			
I. B.	291, 375, 318	251	292
S. C.	179	184, 194, 168, 168	280
M. D.	215	.....	212
B. L.	258	.....	238
E. M.	284	.....	209
B. Requiring Exogenous Steroid			
J. D.	344	.....	288
B. G.	327	.....	306
T. S.	157	180	234

steroid. Changes observed in both groups of the small sample of patients studied appear to be entirely random and give no indication that either subtotal adrenalectomy or minimal steroid therapy exerts any effect upon serum cholesterol concentration.

Short Term Effects of Steroid Dosage upon Blood Pressure of Patients Having Undergone Adrenalectomy for Hypertension. (Figures 4 and 5.) Six experiments have been made in which the minimal hormone requirement of selected patients has been supplemented with additional steroid, desoxycorticosterone or cortisone or both, over a period of some weeks (no other change in regimen being made during the period of observation), and frequent postural blood pressure tests were carried out in an effort to detect direct or early effects of these steroids upon arterial blood pressure. Within the range of dosage used (not more than 100 mg. of cortisone acetate nor more than 4 mg. of desoxycorticosterone acetate were administered per day) no consistent pressor effect could be ascribed to either steroid. Two representative experiments are presented graphically in Figures 4 and 5.

When steroid was reduced below the minimal maintenance therapy, however, marked reduction of arterial blood pressure, as determined with the patient erect, was always observed; a fall of blood pressure measured with the patient lying in a supine position was less consistently observed and much less marked. These observations will be presented in more detail in a report of the clinical features of acute adrenal insufficiency observed under experimental conditions.

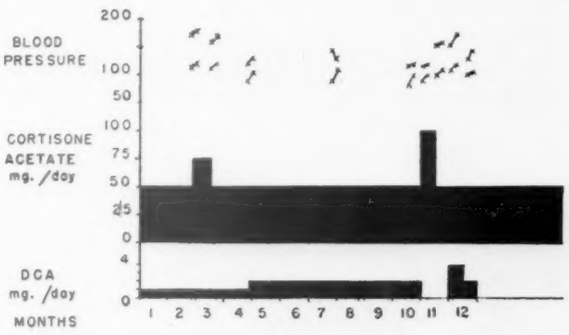

FIG. 4.

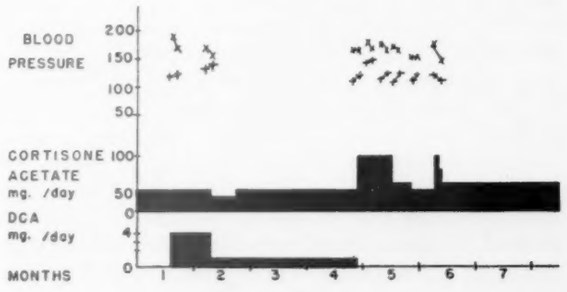

FIG. 5.

FIGS. 4 and 5. Arterial blood pressure was determined twice with the patient in the supine position after ten minutes of rest, then four times at intervals of one minute immediately after the patient assumed the erect position. Upper X's represent the systolic pressure values, lower diastolic. The first of each paired X is the mean of the two values obtained with the patient lying supine, the second of each pair the mean of the four values obtained after the patient stood up.

Regeneration of Adrenal Cortical Tissue Following Subtotal Adrenalectomy. The fact that the minimal hormone requirement has remained stable in most patients having undergone subtotal adrenalectomy for several months following surgical intervention to as long as five years later is a strong indication that substantial regeneration of the remnant is most unusual. Only in one patient have physiologic phenomena been noted which may be interpreted as evidence of some regeneration of adrenal cortical tissue.

The patient, S. W., was subjected to resection of about 90 per cent of the left adrenal and 95 per cent of the right adrenal in August 1950. He was stabilized on minimal hormone therapy of 25 mg. daily of cortisone acetate and 2 mg. daily of desoxycorticosterone acetate; during the first postoperative year and a half

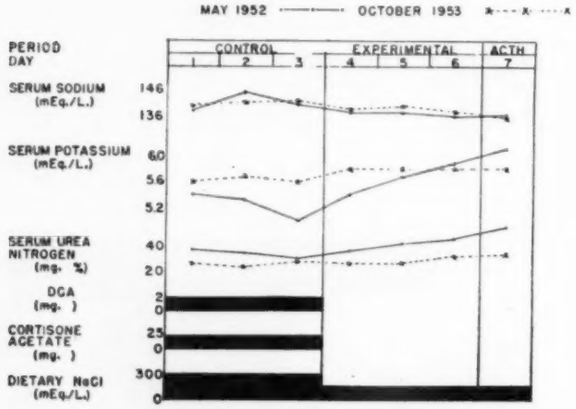

FIG. 6. Evidence suggesting adrenal cortical regeneration in a subtotally adrenalectomized patient (S. W.). The graph is based on data obtained in identical metabolic experiments conducted in the same patient at different times after subtotal adrenalectomy. See text.

he was unable to tolerate further reduction of steroid without marked hyperkalemia associated with azotemia developing. (Fig. 1.) Beginning in November 1952 it became possible to reduce the DCA to 1 mg. daily, cortisone being continued as before, without a clinical abnormality of serious degree developing. In April 1953 the DCA was wholly omitted with relatively moderate rise of serum potassium concentration (to 6.9 mEq./L.) and blood urea concentration (to 33 mg./100 ml.). During that spring two and one-half years following adrenalectomy, this Negro, who had become notably more pigmented following adrenalectomy, experienced a definite generalized depigmentation of the skin with diminution of pigmentary deposits in the palms and palmar creases which occurred over a period of several months. He continues to require 25 mg. of cortisone acetate daily and no desoxycorticosterone.

A study of the effect of withdrawal of all hormone and some dietary sodium chloride had been made in this patient under controlled conditions (diet constant) in the Metabolic Unit in May 1952. The rapidity of onset and the degree of severity of signs and symptoms of adrenal insufficiency which may occur in a patient kept on a standard regimen of this kind reflect the degree of adrenal deficiency present.[17] The study was repeated in October 1953, the experimental conditions being identical with those of the original study, and the serum sodium, potassium and urea were again determined daily. Comparison of the studies (Fig. 6) shows that the rate of increase of the serum concentrations of urea and potassium was distinctly less during the experimental period on the occasion of the second study.

In summary, three types of evidence, depigmentation, late progressive decrease of minimal hormone requirement and decreased rate of development of signs of adrenal insufficiency on hormone deprivation under standard conditions, support the thesis that regeneration of adrenal cortical tissue was occurring at least two years after bilateral adrenal resection. It should be noted that the type of adrenalectomy performed on this patient was unique in our series in that a sizable portion of both adrenal glands was spared at operation.

COMMENTS

Degrees of Human Adrenal Deficiency. The assumption has been prevalent in the past that Addison's disease was the clinical expression of virtually total adrenal deficiency. This assumption rested upon apparently sound pathologic evidence, for in patients with Addison's disease on whom autopsy was performed the quantity of adrenal cortical tissue remaining is always a small and seemingly negligible fraction of the amount normally present.[21,32,40–42] Moreover, the fact that patients with Addison's disease usually fail to show evidence of any consequential response to administration of corticotrophin, when contrasted with the large response exhibited by normal persons, might suggest virtual annihilation of cortical function in such subjects.

Intensive investigation of adrenal physiology during the past twenty years has established the fact that nothing like maximum functioning of the total adrenal cortical tissue ordinarily occurs. A small fraction of the total potential adrenal cortical secretion suffices for ordinary needs, although utilization of a large reserve secretory capacity appears to be necessary for optimal response by the organism to severe stress.[43] It follows that the crucial question concerning the assessment under ordinary conditions of physiologic adrenal cortical inadequacy present in any patient with Addison's disease is this: How does the amount of hormone secreted by the residual adrenal cortical tissue compare quantitatively with the amount of hormone normally secreted by the two intact adrenal cortices under the ordinary circumstances of day-to-day living?

There is reason to suppose, as pointed out in detail elsewhere,[17] that a small residual quantity of adrenal cortical tissue is exposed at all times to intensive stimulation by endogenous

corticotrophin. Although such an overactive remnant can scarcely respond to stimulation with exogenous corticotrophin, its actual secretory production may compare very favorably with that of the intact glands, provided comparison is made in the absence of stressful environmental conditions. It is evident, therefore, that the quantity of secretion produced during life under stress-free circumstances can by no means be supposed to be proportional to the quantity of viable cortical tissue found at necropsy. One might indeed anticipate that the total secretory potential of the adrenal cortical tissue would bear a roughly linear relationship to the quantity of adrenal cortical tissue present postmortem, but correlation of the residual glandular mass with its basal secretory rate during life is possible only on the basis of independent estimates of these quantities by anatomic and physiologic methods respectively.

Methods of evaluating residual adrenal cortical secretion; evidence of residual adrenal cortical function: The corticotrophin test does not distinguish between grades of severe adrenal deficiency. (Table III.) The amount of residual adrenal secretion present in severely adrenal-deficient persons can be estimated by observing the rapidity of onset and the gravity of acute adrenal insufficiency following withdrawal of hormone under standard conditions, but this method[17] of evaluating cortical function is too elaborate to be suitable for a survey of as large a number of patients as are dealt with in this report. The data presented in Tables I and II indicate that a simpler if rougher estimate of residual cortical function is afforded by establishing the minimal hormone requirement of adrenal-deficient subjects.

Supposing, provisionally, that there is for normal adult man a daily, minimal physiologic requirement for cortical hormone which is more or less uniform under ordinary environmental conditions, it is reasonable to assume that exogenous hormone will be required under ordinary conditions only when and to the extent to which the adrenal glands fail to supply this minimal requirement. Ideally, then, there would be a determinable quantity of exogenous hormone which would just support life and health in persons totally deprived of adrenal function. Obviously, a lesser quantity of hormone would have to be administered to persons supplied by their own adrenal glands with much of their requirement, so that the amount of exogenous hormone required by an adrenal-deficient person would directly reflect the severity of the adrenal deficiency.

In practice, of course, the variables affecting any estimate of residual adrenal function from the hormone requirement might prove sufficient to vitiate application of this principle. The amount of cortical secretion which would be required as a minimum might vary from person to person on a constitutional basis; and, as far as these adrenalectomized patients are concerned, the accessory adrenal cortical tissue found so frequently in variable quantity both within and without the abdomen[42,44,45] might obscure the distinction between totally and subtotally adrenalectomized persons. The diversity of environmental conditions affecting outpatients might introduce material variations between persons with respect to the physiologic requirement for adrenal hormone. All these possible sources of individual variation might collaborate to preclude establishment of even a rough standard value for the minimal hormone requirement of persons having undergone total adrenalectomy.

However, from the data given in Tables I and II, which were uncorrected for any such constitutional or environmental factors, it appears that determination of the minimal hormone requirement is not without value in assessing residual adrenal function. It is immediately evident that there is a striking difference between totally and subtotally adrenalectomized patients with respect to the minimal hormone requirements. The seventeen patients having undergone total adrenalectomies have in every instance required substantial amounts of cortisone, and all attempts to withdraw steroid have been followed by manifestations of adrenal insufficiency, even if the intake of sodium chloride was well maintained.* By contrast eight of twenty-seven patients having undergone subtotal adrenalectomy have been maintained without any exogenous hormone, a few have required a steroid dosage comparable to that of the patients having undergone total adrenalectomy, and the others have required smaller quantities.

* In one patient included in the larger series of persons on whom total adrenalectomy was performed in this clinic no sign of adrenal insufficiency developed within seven days following cessation of maintenance steroid therapy. The authors assume that this patient harbors considerable accessory cortical tissue.

From these data two inferences may be drawn. Since all totally adrenalectomized patients required exogenous steroid, it appears that every person has a definite minimal hormone requirement, that is, that no person deprived of all endogenous adrenal hormone can be protected from adrenal insufficiency in the absence of exogenous hormone by any practicable environmental manipulations, even if sodium chloride intake is kept high (10 to 15 gm. daily). In this respect there may be some difference between man and certain of the commonly used experimental animals,[46] although the maintenance of adrenalectomized dogs without exogenous hormone has always been a tour de force requiring environmental and dietary controls which would not be feasible in the clinic.

The second inference is that those subtotally adrenalectomized patients requiring no hormone and probably those requiring notably smaller quantities than any of the totally adrenalectomized patients, must themselves produce appreciable quantities of adrenal hormone. The data do not lend themselves to calculation of precise limits for the minimal cortisone requirement of totally adrenalectomized man but they do show clearly that a totally adrenal-deficient adult can seldom be maintained on as little as 15 mg. of oral cortisone acetate daily. The majority of patients having undergone subtotal adrenalectomy required less exogenous hormone than this, hence they must have appreciable residual adrenal cortical secretion. Maintenance of good health under ordinary circumstances without any steroid therapy whatsoever is unequivocal proof that some endogenous adrenal function is present in such patients. It is unlikely that a person whose hormone requirement is low, say 10 mg. of cortisone acetate daily, is totally deprived of adrenal hormone. But the considerable variation in minimal hormone requirement observed among the patients having undergone total adrenalectomy, from about 15 to 50 mg. of cortisone acetate daily, sufficiently indicates the hazard in exact calculation of the residual adrenal function on the basis of minimal hormone requirement.

Since there is both pathologic and clinical evidence that any consequential regeneration of the adrenal cortex is rare in patients having undergone subtotal adrenalectomy, it is possible to accept the surgical protocol of residual adrenal tissue for this group of patients as a rough estimate of the size of the adrenal remnants during the period of these studies. On this basis one would infer that 2 to 3 per cent of the total adrenal cortical tissue, that is, about the amount which on the average was spared at the subtotal adrenalectomies, is capable of maintaining a sizable fraction, probably 50 per cent or more, of the basal rate of secretion of the normal adrenal cortices. It seems warrantable to infer that a remnant amounting to around 5 per cent of the total cortical tissue is just about capable of supplying the minimal requirement for cortical hormone under the ordinary conditions of daily life. This inference may be compared with that of Barker[41] who estimated from necropsy material that Addison's disease did not occur unless 90 per cent or more of the cortical tissue was destroyed.

Addison's disease and endogeneous secretion of adrenal cortical hormone: When subtotally adrenalectomized patients who did not ordinarily require exogenous hormone were tested, they regularly showed grossly subnormal response to the eight-hour intravenous infusion of corticotrophin (Table III) and unresponsiveness to hypoglycemia (Table IV) and sometimes showed typical melanosis of Addison's disease. (Table I.) Such patients must clearly be regarded as grossly adrenal-deficient, yet in the light of considerations presented in the preceding section, they must also be supposed to have a quantity of endogenously secreted cortical hormone little inferior to that produced by normal glands under basal conditions.

Comparable patients in whom adrenal deficiency is of spontaneous origin are not infrequently encountered. The discovery by Loeb[47] that certain patients with Addison's disease can be kept alive by ensuring adequate intake of sodium chloride constituted a major advance in the management of Addison's disease. Two of the authors' patients who had unequivocal Addison's disease (G. D. and D. A., Table II) were at one time maintained for months on salt alone, and in patient G. D. adrenal insufficiency was first precipitated experimentally by restriction of dietary sodium chloride.

That Addison's disease occurring with appreciable adrenal cortical secretion is in fact the rule rather than the exception is indicated by the quantity of adrenal hormone usually given to maintain patients with this disease. The steroid received by our patients who had

Addison's disease (and presumably by those reported in the literature) does not represent minimal replacement therapy as rigorously determined in our adrenalectomized patients. There is no particular urgency, indeed there may be some disadvantage, in restricting such patients to the minimum steroid therapy required for everyday life, and the figures no doubt represent on the average rather more than the minimum requirement. Even so, it is clear that patients with Addison's disease require smaller quantities of replacement steroid on the average than do patients having undergone total adrenalectomy. (Tables I and II.) The data showing the amount of replacement therapy received by patients with Addison's disease in our clinic are in agreement with the data of other investigaters.[14,48,49] Since there is no reason to suppose that the requirement for adrenal hormone is increased by the concurrence of hypertensive disease, the evidence indicates that the majority of patients with Addison's disease possess appreciable endogenous adrenal secretion.

The preservation of considerable cortical secretion in adrenal-deficient subjects doubtless explains in large part why the urinary excretion of 17-hydroxycorticoids[50] and of 17-ketosteroids and neutral reducing lipids (Table III), as well as plasma 17-hydroxycorticosteroid concentration,[51–52] do not fall consistently to conspicuously low levels in adrenal-deficient persons. Probably no improvement of chemical or biologic methods for the estimation of steroids consequently will ever render simple measurement of blood or urine steroids a satisfactory means of identifying adrenal deficiency. It is safe to say that adrenal-deficient persons differ more strikingly from normal subjects in respect to the magnitude of the adrenal reserve than in respect to the basal rate of secretion of cortical hormone.

The traditional hypostatization of the clinical entity known as Addison's disease should not becloud the fact that one may expect to encounter adrenal deficiency of all degrees, which represents a continuum extending from total absence of adrenal secretion through almost normal basal secretion with little or no reserve to quantitatively normal secretion and reserve. Certain signs of adrenal deficiency may appear if adrenal reserve has been completely or nearly completely wiped out, even if the basal secretory rate may be practically unimpaired, hence no exogenous steroid may be required under ordinary circumstances. On the other hand, patients with detectable although markedly diminished adrenal reserve may perhaps show no overt signs of adrenal deficiency, yet still may be vulnerable to supervention of acute adrenal insufficiency when they are exposed to severe surgical or medical stress. Fortunately the eight hour intravenous corticotrophin test provides a reliable means of establishing the adrenal cortical deficiency of clinical significance, including subclinical cases.

Adequacy of Cortisone as Replacement for Secretion of Adrenal Cortex. The advent in 1949 of cortisone for therapeutic use completely altered the outlook of the adrenal-deficient patient. Desoxycorticosterone, which became available in 1937, had permitted adrenal-deficient persons to survive indefinitely provided serious environmental stress could be avoided, but the life of the patient with Addison's disease was still likely to be "nasty and short." Full therapeutic quantities of this steroid commonly failed to afford a satisfactory return of strength, appetite and well-being. Painful, disabling tendon contractures occurred not infrequently, and the treatment of acute adrenal insufficiency was hazardous and unsatisfactory by modern standards, although adrenal cortical extract was commonly called upon to supplement the actions of desoxycorticosterone.

The 11-17-oxygenated alpha ketolic steroids cortisone and hydrocortisone, compounds very closely related to each other chemically and interconvertible in the human body,[53,54] are, on the other hand, capable of restoring uniformly and completely the lost appetite, weight, strength and well-being of such patients. Taken together with the fact that the striking long-term effects of overdosage with these compounds are practically indistinguishable from those of prolonged overstimulation of the secretion of the normal adrenal cortex by corticotrophin, the remarkable restoration of vitality to the adrenal-deficient organism effected by these steroids suggests that they could afford complete and balanced replacement therapy in adrenal deficiency.[26] It is of course, quite certain that neither cortisone nor hydrocortisone can actually replace the entire secretion of the adrenal cortex in the chemical sense. Although at least one of these compounds is secreted by the adrenal cortex, and hydrocortisone is indeed the predominating substance in the secretory product of the human adrenal cortex, a variety of

other physiologically active compounds are also known to be present in the adrenal secretion.[55] However, its other C-21 compounds have not, with one exception, been shown to possess any striking action which is not possessed either to about the same degree or else much more conspicuously by cortisone and hydrocortisone. The exception is aldosterone, which is many times more potent than either on the basis of weight with respect to promotion of sodium retention and potassium excretion by the kidney.[56] Urinary excretion of aldosterone has also been found to vary inversely with sodium intake,[57] and these facts have led to the widespread supposition that secretion of this steroid by the adrenal is the principal agency by which the sodium balance of the organism normally is maintained.

Nevertheless, the 11-17-oxygenated alpha ketols are not devoid of renal-regulatory activity and convincing evidence, already presented, establishes that salt balance can be maintained throughout a wide range of sodium intake by totally adrenalectomized man maintained on cortisone alone. Still, it may be granted that if environmental conditions are to include salt deprivation, then the modest quantities of exogenous cortisone used for replacement therapy in adrenal-deficient patients would require supplementation with a potent sodium-retaining steroid such as desoxycorticosterone or aldosterone. The question the authors wish to consider here is whether or not cortisone, even when supplemented by small quantities of desoxycorticosterone so that any tendency toward salt waste may be left out of consideration, can be employed in a physiologic quantity which wholly prevents emergence of signs of adrenal insufficiency and at the same time does not give rise to any manifestations of hypercortisonism.

The answer is negative in view of the observations already presented. The quantities of cortisone administered to patients having undergone adrenalectomy as minimal hormone requirement did prevent by definition any gross symptomatic evidence of adrenal insufficiency, but certain signs indicating mild adrenal inadequacy were still demonstrable in all or most of the patients. Such signs included the melanosis of Addison's disease, unresponsiveness to hypoglycemia, positive reactions to the water tests, and in two instances gross abnormalities in plasma electrolytes not preventable by as much as 50 mg. of cortisone acetate per day. These signs were not ordinarily disturbing to the patients, nor would maintenance of mild hypoadrenalism have been considered disadvantageous in the management of these hypertensive patients. However, these manifestations did not represent simple hypoadrenalism resulting from provision of a slightly suboptimal quantity of adrenal hormone, for they were accompanied by certain simultaneous evidence of hypercortisonism. Therefore the entire clinical picture can only be described in physiologic terms as mild endocrine imbalance or dysadrenocorticism. The signs of hypercortisonism comprised two effects well known to result from excess administration of cortisone, namely, stimulation of appetite with resulting weight gain and impairment of glucose tolerance. The authors take these manifestations of hypercortisonism to indicate that normal adrenal secretion under ordinary circumstances provides less 11-17-oxygenated alpha ketolic steroid activity than is furnished by minimal replacement therapy to totally adrenalectomized human subjects.* This inference is entirely consonant with the established fact that the adrenal secretion contains active steroids other than cortisone and hydrocortisone. It is hardly to be doubted that these other steroids circulating in the blood of normal man share with the 11-17-oxygenated steroids in the maintenance of some of the physiologic processes which become deranged in adrenal insufficiency. Their presence in the adrenal secretion permits the adrenal gland in normal man to remain functioning normally with less 11-17-oxygenated steroid being supplied to the subject by his own adrenal glands than must be given to adrenalectomized man as replacement therapy.

* On the basis of our data alone, the possibility would remain open that the abnormal physiologic state of these patients could be ascribed to any factor wherein patients receiving replacement therapy differ from normal persons in whom hormone is supplied by the adrenal glands. One such difference is perhaps insufficiently trivial to dismiss without brief consideration, that is, the provision of hormone on a fixed medication schedule rather than in the normal form of continuous secretion. No plausible chain of causation is evident by which intermittency of hormone administration could lead to the dysadrenocorticism described. In contrast, the inference drawn herein on clinical grounds does provide the needed causative schemes, and indeed one could predict that adrenal-deficient persons treated with cortisone would differ in some manner from normal subjects simply on the basis of the known presence in the cortical secretion of steroids possessing physiologic activity qualitatively differing from that of cortisone and hydrocortisone.

SUMMARY AND CONCLUSIONS

The results of a five-year study of forty-four patients subjected to adrenalectomy (total or subtotal) are presented together with observations of nine patients in whom Addison's disease occurred spontaneously. Cortisone, often but not always supplemented by a small quantity of desoxycorticosterone, was given as replacement therapy.

Steroid therapy is indispensable for the survival of persons who have been subjected to total adrenalectomy but some patients with spontaneous Addison's disease and some subtotally adrenalectomized persons can survive and maintain well-being without it under ordinary circumstances. Such patients apparently enjoy a nearly normal basal rate of adrenal cortical secretion. The survival value of this secretion can be estimated in very crude quantitative terms as corresponding to the average amount of exogenous steroid which will ordinarily just maintain totally adrenalectomized man in good general health (that is, on the average, assuming adequate salt intake, about 30 mg. of oral cortisone acetate daily supplemented by 1 mg. of buccal desoxycorticosterone acetate).

Few patients with Addison's disease are totally destitute of endogenous adrenal cortical secretion. Approximately 5 per cent of the total amount of adrenal cortical tissue normally present will produce enough secretion to sustain life under ordinary circumstances. This quantity of surviving tissue has, however, practically no capacity to increase its basal hormone output, and a patient without ample cortical reserve is imperiled by any stress unless exogenous hormone can then be supplied.

Cortisone can maintain the life of totally adrenal-deficient persons indefinitely and can completely restore strength and well-being but it does not provide an entirely balanced replacement for the missing adrenal cortical secretion of which cortisone and hydrocortisone are only partial constituents. There is no truly "physiologic" quantity of cortisone, that is, there is no dose of cortisone which, on long-term administration to adrenal-deficient patients with or without a small supplement of desoxycorticosterone, will not be accompanied by certain signs of hypoadrenalism or certain manifestations of hypercortisonism or both. The signs of hypoadrenalism commonly encountered in such patients are melanosis, impairment of water diuresis, unresponsiveness to hypoglycemia, and sometimes salt-waste, azotemia and hyperkalemia. The signs of hypercortisonism are polyphagia, weight gain and impairment of glucose tolerance.

In no fundamental respect, either in kind or in degree, can any distinction be drawn between spontaneous and iatrogenic primary adrenal cortical deficiency. The incidence of melanosis has been strikingly lower in patients rendered adrenal-deficient by surgery than in persons with spontaneous Addison's disease, but this difference is probably ascribable to the provision of cortisone to the former group from the time of origin of the adrenal-deficient state. However, although cortisone has antipigmentary properties in adrenal-deficient persons, the adrenal cortex must contain antipigmentary factors other than cortisone and hydrocortisone.

Clinically detectable regeneration of adrenal cortical tissue occurred only once in a series of twenty-seven patients subjected to subtotal adrenal resection.

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Dual Mechanism Regulating Adrenocortical Function in Man*

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ALTHOUGH the anterior pituitary gland is known to govern many of the better understood adrenocortical functions by secretion of corticotropin (ACTH), it does not necessarily follow that it governs all adrenocortical functions. The purpose of the present study is to examine the questions of (1) whether or not the production of aldosterone-like steroids by the adrenal cortex is necessarily associated with the production of hydrocortisone-like steroids, (2) whether or not production of aldosterone is increased when ACTH is administered and (3) whether or not production of aldosterone is diminished by suppression of endogenous ACTH.

METHODS

Repeated observations were made on ten normal volunteer subjects, one patient with hypopituitarism, one with nephrosis, three with cirrhosis, one with amyloidosis and seven with congestive heart failure. Sodium intake was controlled in all studies, and in most studies the subjects received diets which were constant in all respects. During periods of sodium deprivation, dietary sodium was generally maintained at less then 9 mEq. per day. Transitions from periods of low sodium intake to high sodium intake were made abruptly by intravenous infusion of from 2 to 3 L. of physiologic saline solution, followed by the addition of at least 100 mM. of sodium chloride to the daily diet. During periods of ACTH administration, highly purified corticotropin in 16 per cent gelatin vehicle was administered intramuscularly in doses of 100 units every twelve hours. During attempts to suppress pituitary release of ACTH either cortisone acetate or hydrocortisone was administered orally in doses of 50 mg. every six hours.

Complete urine collections for electrolyte and steroid determinations were made daily; until steroid analyses could be performed, the specimens were refrigerated or frozen. In several studies specimens of saliva for sodium/potassium ratio and specimens of blood for eosinophil counts were obtained. Sodium and potassium determinations were made using a flame photometer with lithium as internal standard. As an index of hydrocortisone production urinary 17-hydroxycorticoids were determined by a modification[1] of the method of Silber and Porter.[2]

In preparation for assay of aldosterone-like steroids, the urine was acidified to pH 1 with hydrochloride. It was then extracted continuously with ether for twenty-four hours.† The urine was then extracted continuously with methylene chloride for twenty-four hours. The methylene chloride extract was washed with $\frac{1}{20}$ volume of 0.1 N sodium hydroxide, washed with water, dried over sodium sulfate, and evaporated to dryness with a stream of nitrogen under reduced pressure. The residue was dissolved in ethanol and stored at −7°c. until it could be assayed. It has been shown[3] that after such an extraction procedure, all sodium-retaining activity contained in the final extract behaves chromatographically like aldosterone. Hence the authors have in this discussion used the term aldosterone in referring to sodium-retaining steroids extracted by this method. Aldosterone assay was carried out in adrenalectomized dogs by a method previously described.[4]

RESULTS

Several lines of evidence for the dissociation of aldosterone production from hydrocortisone production were adduced.

Effects of Varying Sodium Intake. In all subjects tested restriction of sodium intake resulted in increased excretion of aldosterone. (Fig. 1 to 6.) Since the degree of sodium depletion was not the same for all subjects it was not surprising that some exhibited much greater increases in urinary aldosterone than others. An abrupt change in regimen from sodium restriction to sodium "loading" was invariably accompanied by an

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† We are indebted to Dr. Maurice M. Pechet for pointing out that extraction of urine with ether can be used to remove the major portion of steroidal contaminants such as free hydrocortisone while removing only minor amounts of aldosterone.

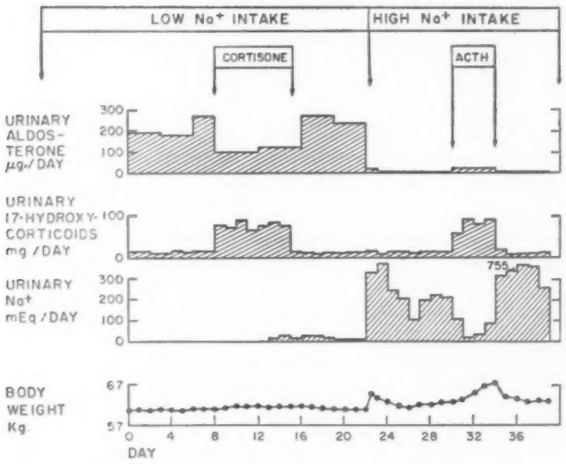

FIG. 1. Patient W. M., twenty-five year old normal man. The effect of cortisone, ACTH, and variations in sodium intake on excretion of aldosterone and 17-hydroxycorticoids (September to October).

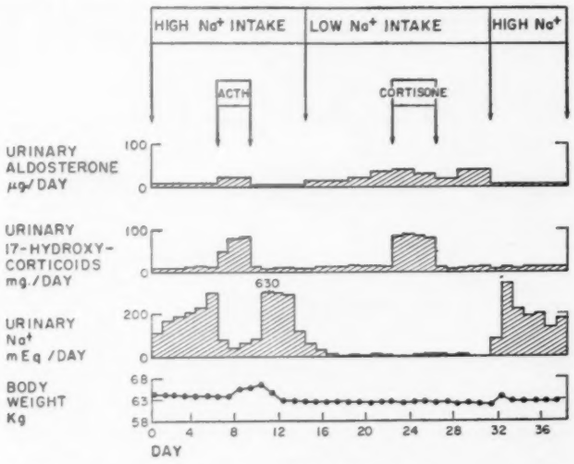

FIG. 2. Patient W. M., twenty-five year old normal man. The effect of cortisone, ACTH, and variations in sodium intake on excretion of aldosterone and 17-hydroxycorticoids (November to December).

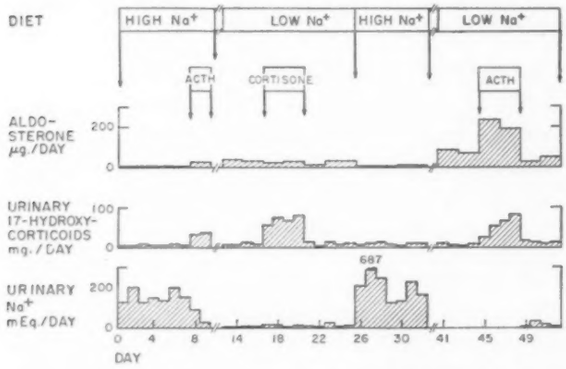

FIG. 3. Patient A. R., nineteen year old normal man. The effect of cortisone, ACTH, and variations in sodium intake on excretion of aldosterone and 17-hydroxycorticoids.

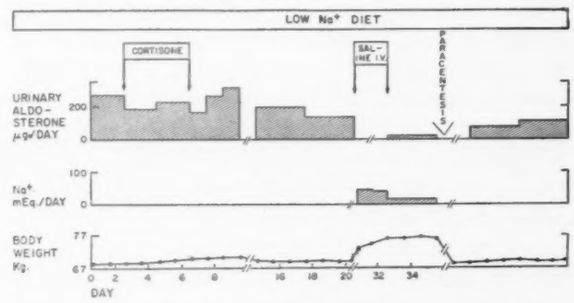

FIG. 4. Patient F. H., forty-four year old man. The effect of cortisone and changes in sodium intake on excretion of aldosterone in a patient with cirrhosis. During the "saline I. V." period 9.4 L. of physiologic saline solution were infused over a period of five days; 7.4 L. of ascitic fluid were removed at the time of "paracentesis."

abrupt fall in urinary aldosterone. Excretion of 17-hydroxycorticoids was not influenced by sodium intake. This suggests that sodium deprivation as a stimulus to aldosterone production is not mediated by ACTH.

During periods of sodium deprivation salivary sodium/potassium ratios became depressed, presumably in response to increased circulating levels of aldosterone.[5] Significant changes in circulating eosinophils did not occur. Eosinophils were not expected to show a change, of course, since other evidence of increased hydrocortisone secretion was absent and since aldosterone itself has relatively slight eosinopenic activity.[6,7]

Adrenal Function in Diseases Characterized by Edema Formation. Patients in whom edema forms due to congestive heart failure, cirrhosis or nephrosis exhibit characteristic distortion of adrenocortical function. (Fig. 7.) Whereas 17-hydroxycorticoid output in such patients is usually normal or slightly low, aldosterone output is high even when sodium intake is not restricted. This suggests that in certain pathologic conditions a stimulus to the production of aldosterone develops which does not affect the production of hydrocortisone.

Effect of ACTH. A total of fourteen courses of ACTH were administered to five endocrinologically normal subjects. (Figs. 1–3, 5, 8–11.) The duration of treatment with ACTH varied from one to eight days. These studies with ACTH included subjects on both high and low sodium regimens. With all fourteen courses of treatment, administration of ACTH was accompanied by

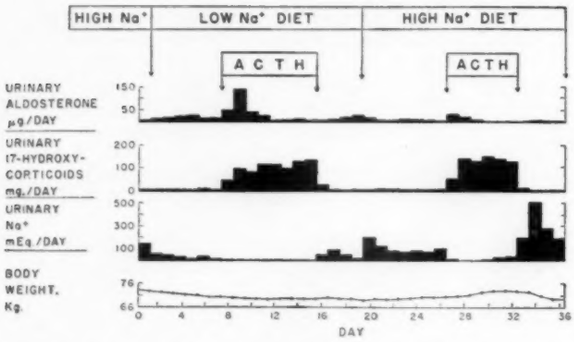

FIG. 5. Patient R. K., twenty-four year old man. The effect of ACTH on urinary aldosterone and 17-hydroxycorticoids in a normal subject maintained on a sodium intake, first, of 15 mEq. per day and, later, 100 mEq. per day.

large increases in 17-hydroxycorticoid excretion and, with equal consistency, by relatively smaller increases in aldosterone excretion. On the average, the increases in 17-hydroxycorticoid excretion were tenfold, whereas the increases in aldosterone excretion were twofold. The ACTH-induced increase in aldosterone excretion was not sustained beyond the initial two to four days of treatment. As illustrated in Figure 5, with more prolonged administration of ACTH, aldosterone excretion fell to levels below those observed in the pre-ACTH periods. This late fall in aldosterone excretion was not accompanied by any diminution in the high levels of urinary 17-hydroxycorticoids.

The abrupt withdrawal of ACTH following a short course of treatment was in every instance followed by a fall in urinary aldosterone to extremely low levels. This fall in urinary aldosterone was always accompanied by some sodium diuresis.

Effect of ACTH-Suppressing Steroids. Since cortisone in doses of approximately 25 mg. every six hours will induce adrenocortical atrophy[8] and unresponsiveness[9] in human subjects, it is reasonable to assume that such doses are effective in suppressing secretion of ACTH. It is noteworthy, therefore, that the much larger doses of cortisone acetate employed in this study (50 mg. every six hours) were not very effective in suppressing aldosterone excretion of subjects on low sodium intake. (Figs. 1–4.) In three studies aldosterone excretion fell slightly, and in one study it rose slightly during administration of cortisone. In three of four studies a transient fall occurred in urinary aldosterone immediately following withdrawal of cortisone.

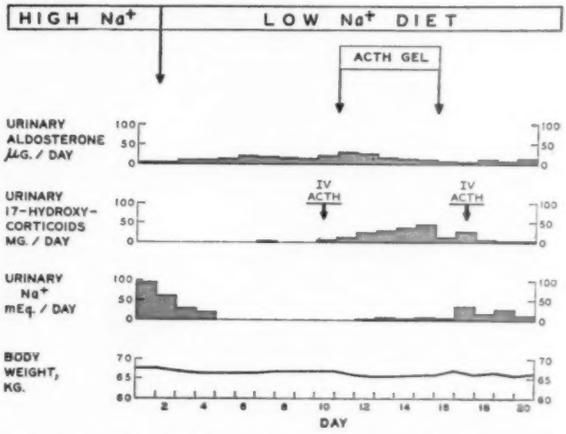

FIG. 6. Patient J. M., twenty-nine year old man. The effect of ACTH and changes in sodium intake on urinary aldosterone and 17-hydroxycorticoids in a patient with hypopituitarism. "I. V. ACTH" denotes intravenous infusion of 40 units of ACTH over an eight hour period. In our series of normal adult subjects 17-hydroxycorticoid excretion values ranged from 20 to 40 mg. per day during such standardized stimulation.

Hydrocortisone in doses of 50 mg. every six hours failed to suppress aldosterone excretion to any marked degree in subjects on low sodium intake. (Figs. 8, 12.) It appears, therefore, that cortisone and hydrocortisone are much less effective than a liberal sodium intake in suppressing aldosterone excretion.

Aldosterone Production in Hypopituitarism. Subject J. M. was a twenty-nine year old man in whom hypopituitarism had been well established. Gross impairment of the adrenal hydrocortisone-secreting mechanism in this patient was indicated by the very low levels of plasma and urinary 17-hydroxycorticoids. (Fig. 6.) The response to exogenous ACTH was characteristic of that seen in patients with adrenocortical atrophy secondary to pituitary hypofunction in that only a minimal rise in plasma and urinary 17-hydroxycorticoids occurred during the initial day of treatment with ACTH, with stepwise increases to normal with six successive days of treatment. Although the pituitary gland of this patient was not functioning adequately to maintain the adrenal hydrocortisone-producing mechanism, no gross impairment of the aldosterone-producing mechanism occurred. During sodium deprivation increased quantities of aldosterone were excreted in this patient, and the urinary sodium fell to less than 1 mEq. per day.

This case confirms in another manner the independence of hydrocortisone and aldosterone-

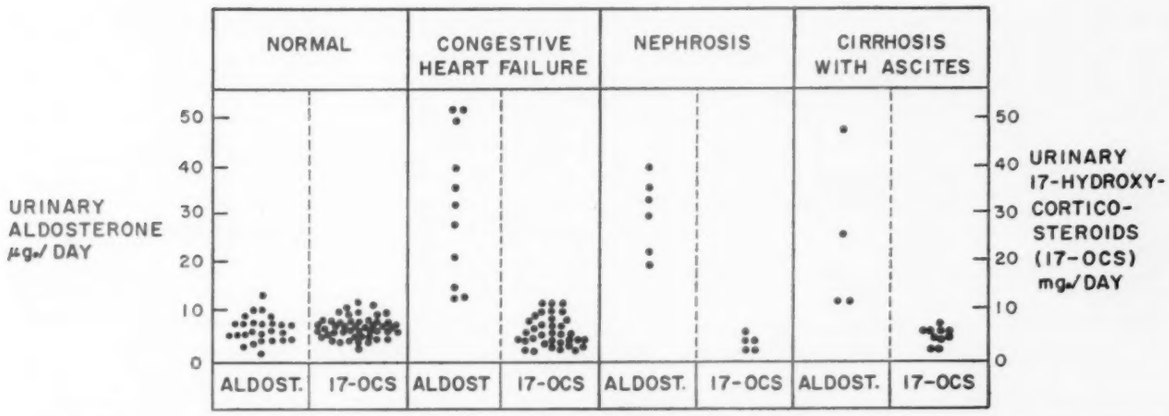

FIG. 7. The range of values encountered for aldosterone and 17-hydroxycorticoid excretion in normal adult subjects and in a series of patients exhibiting edema due to congestive heart failure, nephrosis or cirrhosis. All values were obtained while subjects were receiving a liberal sodium intake but were not receiving hormone therapy.

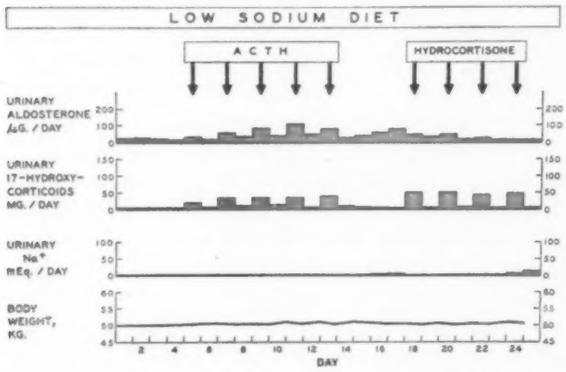

FIG. 8. Patient E. M., nineteen year old normal woman. Aldosterone excretion during treatment on alternate days with ACTH and during comparable treatment with hydrocortisone.

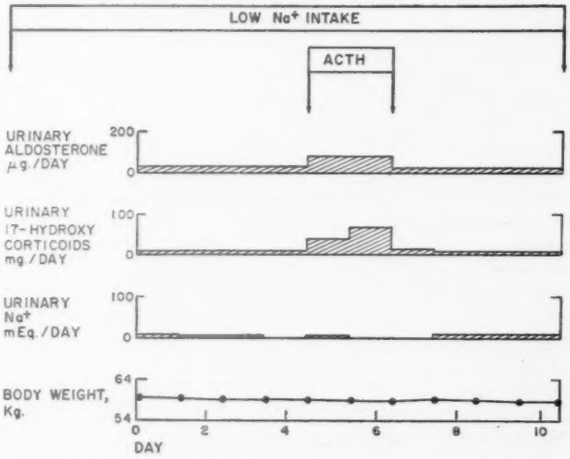

FIG. 9. Patient W. M., twenty-five year old normal man. The effect of ACTH on steroid excretion (February).

producing mechanisms. It indicates that aldosterone production does not require stimulation by ACTH and, furthermore, does not depend upon an intact pituitary for its physiologic control.

COMMENTS

The findings of the present study supplement previous work showing that sodium deprivation is a stimulus to increased aldosterone excretion[10] and that aldosterone excretion is frequently increased in patients with congestive heart failure,[10,11] nephrosis,[10,13] and cirrhosis.[10,14] That destruction of the pituitary body does not lead to complete cessation of secretion of aldosterone in the dog,[15] the rat,[16] and man[12,17] has been reported previously.

The consistent observation in the present study that ACTH induces an increase in aldosterone excretion is in agreement with a report by Gordon, et al.[12] based upon a study in man, as well as studies on the rat reported by Singer and Stack-Dunne.[16] Contrary findings have been reported by Luetscher and Johnson,[10] and by Venning et al.[18] The increases in aldosterone excretion which occur in response to large doses of ACTH are small when compared with the increases which can be brought about by severe sodium deprivation. The effect of ACTH on aldosterone excretion is biphasic; an initial increase is followed by a decrease. To explain this, one may postulate that ACTH has some "tropic" influence on the adrenal gland which favors secretion of aldosterone, but the concomitant secretion of large quantities of other corticoids leads to cumulative physiologic effects (for example, sodium retention, potassium loss,

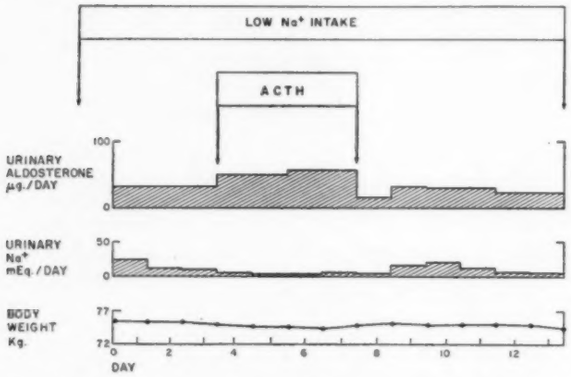

FIG. 10. Patient J. B., twenty-six year old man. Effect of ACTH on aldosterone excretion in a patient with familial amyloidosis.

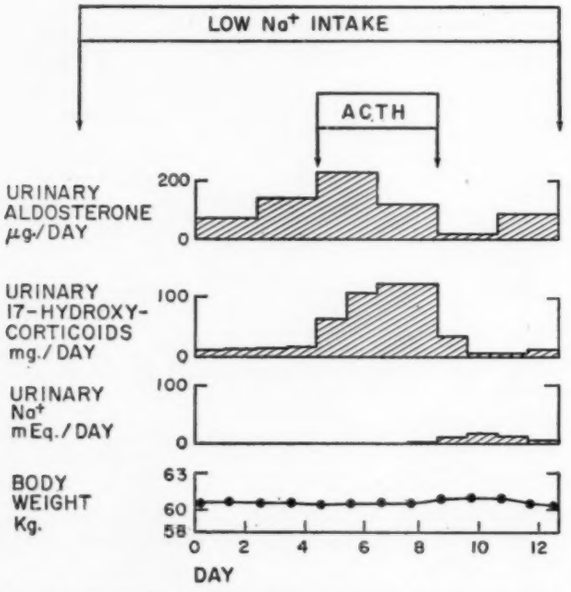

FIG. 11. Patient W. M., twenty-five year old normal man. Effect of ACTH on steroid excretion (January to February).

extracellular fluid expansion) which oppose aldosterone secretion. ACTH should be regarded, then, as an ancillary agent capable of producing transient changes in aldosterone secretion, whereas the prime factor determining the level of aldosterone secretion is related to fluid and electrolyte metabolism. It is of interest that in various studies the percentage increase in aldosterone excretion induced by ACTH was relatively constant, whereas the absolute increase was clearly a function of the pre-ACTH levels of aldosterone excretion. (Figs. 3 and 5.) If the increase in excretion of sodium-retaining corticoid during treatment with ACTH were due to

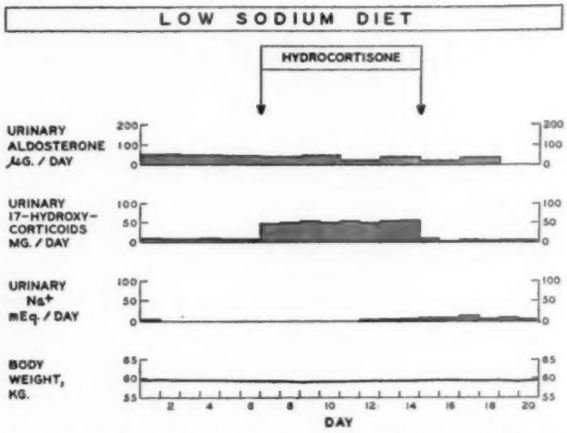

FIG. 12. Patient V. B., nineteen year old normal man. Effect of hydrocortisone on aldosterone excretion.

some steroid other than aldosterone (corticosterone, for example), then one would expect the absolute magnitude of the increase to be unaffected by such factors as the amount of sodium in the diet.

The "unitarian" concept that adrenocortical function is under the sole control of pituitary adrenocorticotropic hormone has become untenable. Several lines of evidence indicate that at least one adrenocortical function, the secretion of aldosterone, is regulated by some other mechanism. Hydrocortisone secretion is apparently regulated strictly by ACTH, while aldosterone secretion is affected relatively little by either increases or decreases in ACTH. Aldosterone secretion is extremely sensitive to changes in body fluid and electrolyte content, whereas hydrocortisone secretion is affected hardly at all by these changes.

Such separation of adrenocortical functions in the rat was recognized many years ago in the cytologic studies of Greep and Deane.[19] These investigators were able to change the histochemical appearance of zona glomerulosa in rats by treatment with desoxycorticosterone acetate but not by hypophysectomy or by treatment with corticosterone. They could, however, alter the appearance of zona fasciculata by hypophysectomy or by treatment with corticosterone. Whether aldosterone and hydrocortisone are secreted by different zones of the human adrenal cortex is, of course, unanswered by the present study.

Although it is clear that aldosterone is not ordinarily regulated by ACTH, it remains for further studies to elucidate the precise nature of the regulatory mechanism. Some of the condi-

tions which stimulate aldosterone production are (1) loss of extracellular fluid volume, whether it is a result of simple dehydration[20] or sodium depletion, (2) potassium loading[20,21] and (3) certain diseases such as congestive heart failure, cirrhosis and nephrosis. Whether or not the multiple factors which now appear to influence aldosterone production will ultimately be shown to operate through a "final common pathway" remains to be determined.

Recognition of the fact that aldosterone secretion is not dependent upon ACTH makes it easier to understand that patients with hypopituitarism, although displaying inferior tolerance to trauma, infections, and fasting, nevertheless often survive sodium deprivation very well. This ability to conserve sodium is not shared by patients with Addison's disease. Dilution hyponatremia does, of course, occur in patients with hypopituitarism, due to their inability to excrete a water load readily; this defect is remedied by treatment with cortisone.[22]

It is of interest that at least one form of hyperadrenocorticism (that is, increased aldosterone output) frequently occurs without giving rise to any of the more common signs of hyperadrenocorticism. An abnormal increase in aldosterone production occurs with great frequency in patients with congestive heart failure, cirrhosis and nephrosis, and probably contributes significantly to the edema forming propensity of such patients. Yet this variety of adrenal hyperfunction is not associated with the clinical or laboratory stigmas of Cushing's syndrome. Specifically, there is no characteristic alteration in 17-hydroxycorticosteroid or 17-ketosteroid values or in eosinophil counts in patients in whom large quantities of aldosterone are produced.

SUMMARY

Secretion of aldosterone and secretion of hydrocortisone by the human adrenal cortex appear to be regulated by distinctly different mechanisms, as shown by the following observations.

1. Sodium deprivation results in large increases in aldosterone output but does not appreciably affect 17-hydroxycorticoid output.

2. Certain diseases (congestive heart failure, cirrhosis and nephrosis) are characterized by an increase in aldosterone output without clinical or laboratory evidence of more general hyperadrenocorticism.

3. Administration of ACTH results in relatively large increases in hydrocortisone (17-hydroxycorticoid) output but results in only comparatively small increases in aldosterone output.

4. Suppression of ACTH release by administration of cortisone or by damage to the pituitary reduces hydrocortisone secretion to minimal amounts but has relatively little effect on secretion of aldosterone. Thus it appears that the secretion of aldosterone is responsive to changes in water and electrolyte metabolism, whereas the secretion of hydrocortisone is regulated by production of ACTH.

Acknowledgment: The authors wish to express their gratitude to Miss June Richard and Mrs. Gaynelle Greene for their technical assistance.

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Review

The Clinical Significance of the Analysis of Serum Protein Distribution by Filter Paper Electrophoresis*

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THE development of technics for the electrophoretic separation of serum on filter paper followed by estimation of the concentration of the separated protein fractions by staining with acid dyes[1,6] has made possible the widespread application of the electrophoretic technic and its high degree of resolution in protein fractionation to clinical problems. Unfortunately, much of the quantitative work with paper electrophoresis so far reported has been carried out with methods which were not satisfactory for the quantitative determination of separated, denatured protein fractions,[7] because of a nonlinear relationship between protein concentration and dye uptake,[8] or the use of results obtained by direct scanning of stained proteins on paper strips without correction for the nonlinear relationship between dye concentration on paper and scanner response.[9] A recently described method for the quantitative determination of electrophoretically separated proteins on paper with the dye bromphenol blue yielded a linear relationship between protein concentration and dye uptake and a scanner response which is linear for the concentrations of dye bound by normal globulin fractions and which is capable of correction for the albumin fraction.[10] With the use of this procedure it has been possible to obtain results which, while not as accurate as those obtained by the moving boundary electrophoretic technic, appear to be of sufficient reliability and reproducibility for most purposes and are not subject to serious errors of the kind so far described.

No attempt will be made here to review completely the voluminous literature on the application of electrophoretic technics to the study of plasma and serum proteins in various diseases. Most of the important findings have been summarized by Gutman[11] in a review on the results obtained with the moving boundary method and on the relationship between the salting-out and electrophoretic methods of protein fractionation. Other recent publications include those by Luetscher,[12] Fisher[13] (reviews), Lever[14] (emphasizes dermatologic conditions), Flynn[15] (qualitative estimation of abnormalities in paper electrophoretic patterns), Koïw et al.,[16] Paton et al.[17] and Macheboeuf et al.[18] (paper electrophoresis), Coryell et al.[19] (pregnancy), Arends et al.[20] (Hodgkin's disease and lymphoma), Rundles et al.[21] (leukemia), Ropes et al.[22] and Hunt[23] (rheumatoid arthritis), Squire,[24] Fisher et al.[25] and Stickler et al.[26] (nephrotic syndrome), Hoch-Ligetti et al.[27] (surgery), Mahaux and Koïw[28] (myxedema), Bruton[29] (agammaglobulinemia), Osserman and Lawlor[30] (multiple myeloma), Wilson and Lubschez[31] (rheumatic fever), Satoskar et al.[32] (hepatitis), Stauber[33] (parasitology), and Kay[34] (paper electrophoresis in the differential diagnosis of ascites). Some of the quantitative results obtained with paper electrophoretic technics by these authors are uncertain because of the methods used, although the general patterns of abnormality in various diseases are probably correct.

The study reported herein was carried out in order to obtain data on the range of normal values for distribution of serum proteins as

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measured by the paper electrophoretic technic described previously, on the type, incidence and diagnostic significance of abnormalities of this distribution in disease, and on the usefulness of this technic as a clinical laboratory procedure.

MATERIALS AND METHODS

Electrophoretic separation and staining of proteins on filter paper were carried out as previously described.[10,35] Bromphenol blue staining solution was freshly made each week and used for staining no more than twenty-five strips per liter. The amounts of dye bound by each of the separated protein fractions were estimated either by elution or by direct optical scanning of the oiled strips using a continuously recording instrument[36] or a similar instrument constructed in such a way that the optical density and integral curves are recorded simultaneously by a two-pen recorder.* Light from a tungsten lamp was passed through a Bausch and Lomb second order interference filter with a peak transmission at 604 mμ., a Corning 3,385 cut off filter, and a 2 mm. slit before passing through the paper.

The percentage contribution of each protein fraction to the total pattern area was read directly from the rise in the integral curve for each component, using a proportional ruler graduated from 0 to 100 per cent. The integral distances obtained for the albumin fractions were corrected for deviation resulting from non-linear relationship between optical density and concentration of dye on paper by reference to a standard curve obtained by elution of a group of normal and pathologic serum patterns. Under the scanning conditions described, this correction was close to 2.0 for the greater part of albumin concentrations encountered; this results in an increase to 65 to 70 per cent from the uncorrected value of 50 to 55 per cent for the fraction of the total area due to albumin in normal serum. As previously reported[10] no correction is necessary for measurement of dye bound to the less concentrated globulin fractions. No attempt was made to correct scan values obtained for concentrated abnormal globulins such as those encountered in serum from patients with multiple myeloma. Such proteins result in a high concentration of dye distributed over a small area of paper and are best measured by elution if an accurate estimate of their concentration is desired. Unless otherwise stated, all the data were obtained with the direct scanning technic. No correction was made for albumin "tailing" which amounts to 3 to 5 per cent of the albumin present in normal serum.

The reproducibility of repeated determinations on the same sample under the conditions described has been reported previously.[10] Standard deviations for the percentage of the total dye bound by the albumin fraction averaged slightly over 2 per cent when determined by elution or by direct scanning without correction. After correction the values for the various fractions obtained by scanning and by elution agree satisfactorily and the standard deviations of the corrected scan values for albumin average about 3 per cent of the total dye bound with correspondingly smaller values for the other fractions.

Results are expressed in terms of bromphenol blue dye binding capacity of the separated fractions. No attempt has been made to convert the results to units of refractive index, nitrogen content, dry weight or other terms. Such a conversion would require the use of different conversion factors for each protein fraction[10] and, even if accurate conversion factors were determined for each of the fractions of normal serum, it is unlikely that the same factors would be valid for proteins of different composition in abnormal serum.

Total protein was determined by the biuret method[37] using as a standard bovine albumin, the concentration of which was determined by micro Kjeldahl nitrogen analysis using the factor 6.25 to convert to protein concentration.

Completed results on each sample were transferred to Keysort punch cards for sorting and analysis.

RESULTS

Paper electrophoretic analyses of serum protein distribution were carried out on serum samples from 3,000 hospital admissions as part of the routine initial laboratory work-up on these patients, excluding only those patients admitted to psychiatric wards. From the last 2,300 of these analyses, 1,516 cases on which complete data and final discharge diagnoses were available were selected for further study. Of this group, 325 represented obstetric cases, forty-one were cord bloods obtained from newborn infants, and the remainder a random sampling of hospital admissions including 185 specimens which were later used for the determination of normal values, as will be described. Results were analyzed first by disease to determine incidence and nature of serum protein abnormalities in various disease groups, and then by abnormality to determine the types of disease and the diagnostic significance associated with each type of abnormal finding. The type of pattern obtained and some abnormal patterns evident on inspection of the stained strips are shown in Figure 1.

In the interpretation of data to be presented

* We are greatly indebted to Mr. Saul R. Gilford and Mr. Melvin Martin of the National Bureau of Standards for the construction of this instrument.

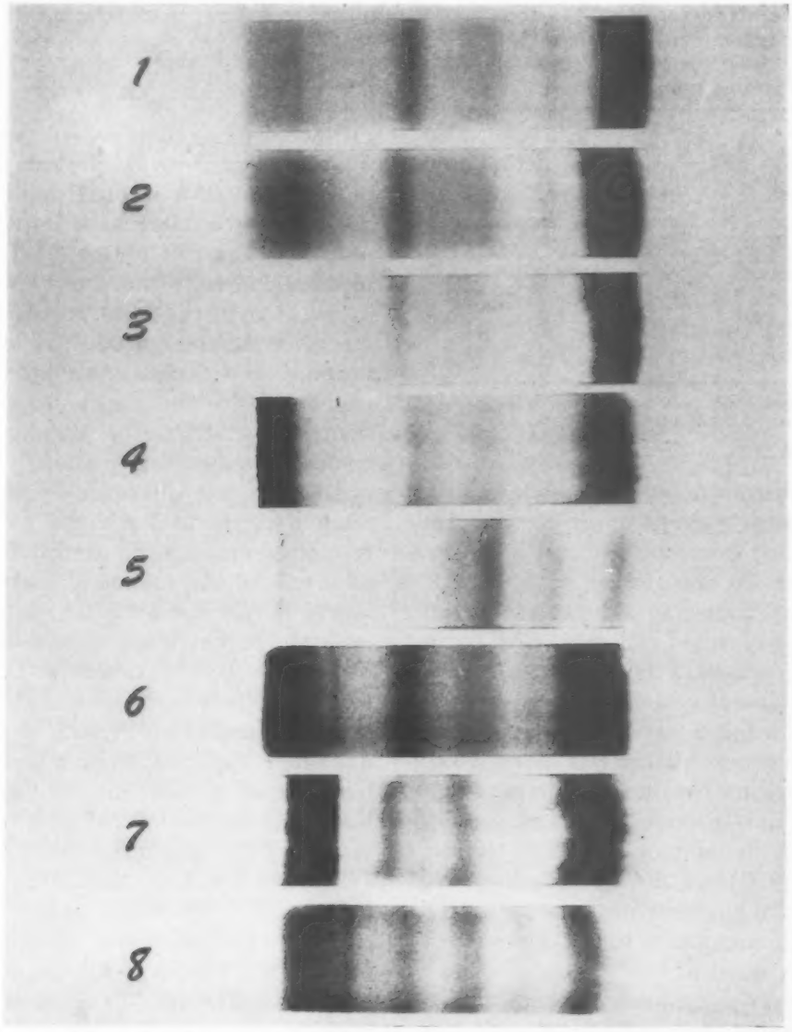

FIG. 1. Examples of abnormalities in serum protein distribution which are evident on inspection of the electrophoretic pattern. *1*, normal; *2*, infectious mononucleosis; *3*, hypogammaglobulinemia; *4*, leukemia (type undetermined); *5*, nephrotic syndrome; *6*, infectious hepatitis; *7*, multiple myeloma; *8*, sarcoidosis.

two points should be kept in mind. First, the concentration of each serum protein fraction is expressed in terms of per cent of the total protein rather than as absolute serum protein concentration. This is done to avoid the introduction of an error caused by multiplying per cent values obtained in one set of units (dye binding capacity) by total protein values in another set of units (biuret value [this applies equally to results obtained with the moving boundary method, unless total protein concentration is measured in each case by either refractive index increment itself or by dry weight, which has been shown to be directly proportional to the refractive index increment for a variety of different proteins[38]]), and also to avoid variations which would occur as a result of changes in total protein concentration. Such changes, resulting from transient hemoconcentration or hemodilution due to postural changes,[39,40] state of hydration, and so on, would lead to apparent changes in the absolute concentration of protein fractions in the absence of any change in the protein distribution pattern. As a consequence of expressing results in terms of per cent, any change in the concentration of one fraction will lead to complementary changes in the percentage distribution of all other fractions. This is of

TABLE I
PROTEIN DISTRIBUTION OF POOLED NORMAL SERUMS DETERMINED BY ELUTION OF PATTERNS STAINED WITH BROMPHENOL BLUE *

No. of Samples of Pooled Serums	Albumin (%)	Alpha$_1$ Globulin (%)	Alpha$_2$ Globulin (%)	Beta Globulin (%)	Gamma Globulin (%)
40.....	70.8	2.7	5.7	8.3	12.6
10 (No. 1)....	70.8	2.5	7.8	8.7	10.3
10 (No. 2)....	71.1	2.6	7.0	8.2	11.2
10 (No. 3)....	69.9	2.8	7.5	8.8	11.0
Average....	70.6	2.7	7.0	8.5	11.3

* Values are means from at least two determinations on each sample. Serums were obtained from predominantly male blood donors at time of bleeding.

practical importance only in those cases in which a decreased concentration of albumin results in increased per cent values for all the globulin fractions. For this reason in the evaluation of results a globulin fraction was considered to be abnormally elevated only if the albumin concentration was normal or, in the presence of a decreased albumin concentration, only if the other globulin fractions were all normal or the total protein concentration was increased.

Second, the results obtained by paper electrophoresis are not directly comparable with those obtained by the moving boundary technic since protein-bound lipid and carbohydrate are not measured by the former procedure and the ratio of dye binding capacity to refractive index increment varies for different proteins.[10] Since lipid and carbohydrate are bound almost entirely by globulins, and because albumin has the highest dye binding capacity of any of the protein fractions of normal serum,[10] the net result of these differences is that the concentration of albumin in normal serum is higher when measured by paper electrophoresis than when measured by the moving boundary technic.

Normal Values. In Table I are shown the values for protein distribution obtained by analysis of four different pools of serum from presumably healthy, non-professional blood donors. These values were obtained by elution of dye from the individual protein bands; corrected scan values on the same samples were not significantly different. The 70 per cent value obtained for the albumin fraction of these serum pools may be compared with the 55 per cent value obtained for pooled human plasma by Armstrong, Budka and Morrison[41] by the moving boundary method in terms of refractive index (59 per cent if the value for fibrinogen, which is not present in serum, is omitted). The reasons for this difference have already been discussed.

Since results obtained with pooled serum and plasma do not provide the range of variation to be expected in a normal population, they are not entirely suitable as a standard for clinical purposes. For this reason, and to rule out the likelihood of error because of possible alterations in protein distribution caused by pooling of serum or differences between blood donor and hospital populations, the protein distribution was determined in serum from a group of patients hospitalized for various reasons which could be reasonably assumed not to be associated with any abnormality of serum proteins. Such a group was selected by assembling the results of analyses on serum from all patients with one of the following four discharge diagnoses: (1) no disease found, seventy; (2) minor injuries, twenty-four; (3) localized mild skin diseases, forty; (4) deafness (excluding trauma and otosclerosis), fifty-one. Mean values for protein distribution in each of these groups did not differ appreciably from the others, indicating that no abnormality in one group was influencing the mean value obtained for the entire series. The values obtained from this group of 185 cases are summarized in Table II. It is evident that the mean values for protein distribution in this series do not differ greatly from those obtained with the pooled serum, the greatest difference occurring in the albumin fraction which is 68.9 per cent compared with 70.6 per cent in the former series.

If it is assumed that the distribution of normal concentrations of various protein fractions follow a normal distribution curve, then 95 per cent of all normal values will be included within a range of two standard deviations above and below the mean normal value for each fraction. The range of normal values used in this study was defined by these limits, keeping in mind that one of twenty normal subjects will fall outside this range. This range of values, rounded off to the nearest whole number, is also shown in Table II. The data presented on abnormal serum have been compared with this group of normal values, and the references to significance in statistical comparisons refer to comparisons with this group. The significance of such comparisons was measured by the "t test" and is expressed in terms of "p," the probability that the differ-

TABLE II

NORMAL VALUES FOR PROTEIN DISTRIBUTION OBTAINED BY SCANNING PAPER ELECTROPHORETIC PATTERNS OF SERUMS FROM 185 PERSONS

Values	No. of Persons	Albumin (%)	$Alpha_1$ Globulin (%)	$Alpha_2$ Globulin (%)	Beta Globulin (%)	Gamma Globulin (%)	Total Protein (gm./100 ml.)
Average	185	68.9	2.9	7.3	9.0	12.0	7.3
Standard deviation	...	4.2	0.9	1.5	1.9	2.5	0.7
Standard deviation of mean	...	0.3	0.1	0.1	0.1	0.2	0.1
Normal values: mean ± 2 standard deviations	...	60–77	1–5	4–10	5–13	7–17	5.9–8.7
Men	163	68.9	2.9	7.2	9.0	11.9	7.3
Women	22	68.5	3.2	7.7	8.5	12.0	7.1

ence between the two groups being compared is due to chance alone.

The mean values for protein distribution according to sex in the same group are also shown in this table. Values for the two groups are closely comparable and indicate there is little or no difference in the electrophoretic distribution of proteins between men and women.

The mean values for protein distribution by age are shown in Figure 2. Although the majority of cases are in the younger age group, there are enough cases in the older groups to indicate that there is no great change in the electrophoretic distribution of serum proteins over the age of fifteen.

Serum Protein Abnormalities in Disease. Infections. The most frequent and striking abnormality in the protein pattern found in analyses of serum from 187 cases of infectious disease was an elevation in the level of $alpha_2$ globulin. Thirty-nine persons displayed values greater than the range of normal for this protein fraction and in the entire group 141 of the 187 patients had values above the average normal value. The mean $alpha_2$ globulin level was 8.7 per cent compared with the normal mean of 7.3 per cent. Almost as many patients had abnormally decreased albumin concentrations, with thirty-seven values below the normal range, while only fifteen patients had abnormally elevated gamma globulin levels.

The mean values for distribution of serum proteins in twenty-two cases of pulmonary tuberculosis are shown in Table III. The albumin and gamma globulin fractions are significantly ($p = <0.001$) below and above normal, respectively. There is also an elevation in the level of $alpha_2$ globulin; the small increases in $alpha_1$ and beta globulin reflect only the fall in the albumin fraction and do not indicate an appreciable rise in the concentration of these fractions. The changes from the normal pattern in this group correspond closely to those reported by

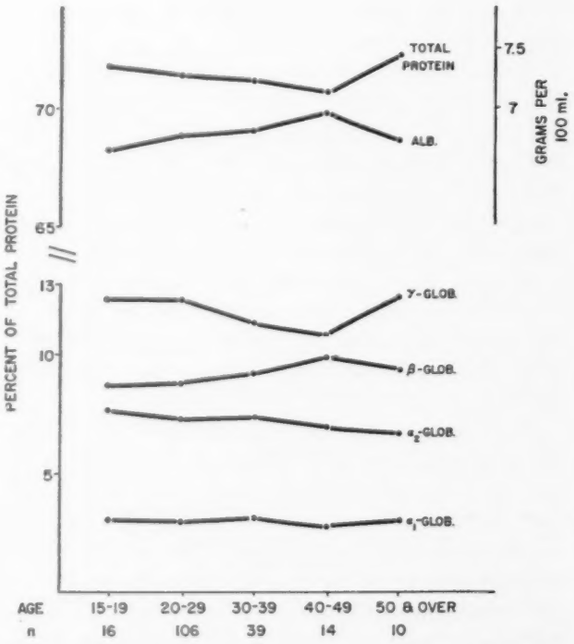

FIG. 2. Distribution and concentration of serum proteins in normal subjects according to age.

TABLE III

SERUM PROTEIN DISTRIBUTION AND CONCENTRATION IN PERSONS WITH INFECTIOUS DISEASES

	No. of Persons	Albumin (%)	Alpha$_1$ Globulin (%)	Alpha$_2$ Globulin (%)	Beta Globulin (%)	Gamma Globulin (%)	Total Protein (gm./100 ml.)
Pulmonary tuberculosis	22	59.6	4.0	9.5	9.9	17.0	7.0
Pneumonia	11	65.0	4.1	8.8	9.7	12.4	6.9
Bronchopneumonia	12	64.3	3.9	10.0	9.6	11.9	6.7
Pyelonephritis	11	59.5	4.7	10.0	10.5	15.3	7.0
Standard deviation	..	6.1	1.5	3.1	3.3	3.7	0.9
Significance (p)	..	0.001	0.001	0.001	0.05	0.001	None

Seibert et al.[42] for their cases of "moderately advanced active pulmonary tuberculosis," although the actual values are not the same because of the different units used to express protein concentration.

In the individual analyses on these patients the albumin concentration was below the average normal value in every case and below the normal range in ten cases, with values ranging down to 44 per cent. The gamma globulin level was above the average normal value in all but two cases and abnormally elevated in five, with values up to 34 per cent. The alpha$_2$ and beta globulin fractions were abnormally elevated in three and two cases, respectively, and total protein concentration was below normal in three cases.

The mean values for concentration of various protein fractions in a group of eleven cases of pneumonia, only two of which were definitely diagnosed as pneumococcal, are shown also in Table III. There is a small decrease in the mean albumin concentration but little change in the other fractions except for a slightly elevated alpha$_2$ globulin. The albumin was abnormally decreased in two cases and the alpha$_2$ globulin elevated in three cases, with ten of the eleven cases showing values above the mean normal value. In one case total protein concentration was decreased. In no case was gamma globulin concentration increased.

Of twelve cases of bronchopneumonia, all but one exhibited levels of alpha$_2$ globulin above the normal mean and in seven cases this was outside the range of normal values. In addition, one albumin value and one total protein value were abnormally low. The average values for this disease (Table III) do not differ appreciably from those for pneumonia except for a somewhat greater alpha$_2$ globulin concentration.

In thirteen cases of bronchitis, the alpha$_2$ globulin was abnormally elevated in two cases and above the average normal value in eleven cases. The albumin concentration was decreased to 53 per cent in one case.

In eleven cases of bronchiectasis albumin concentration was decreased in two cases and gamma globulin and alpha$_1$ globulin were each increased in one case.

Of the thirty-four patients with infections of the upper respiratory tract (including thirteen cases of tonsillitis and adenoiditis, and thirteen cases of otitis media), three had abnormally low albumin concentrations, two had elevated alpha$_1$ globulin, and one each had decreased alpha$_2$ and beta globulin concentrations.

The only abnormality encountered in five patients with osteomyelitis was one albumin concentration of 59 per cent.

The mean values for concentration of protein fractions in eleven cases of pyelonephritis are shown in Table III. The mean albumin concentration of 59.5 per cent is considerably below normal and accompanied with an increase in all the globulin fractions, which is most marked for the alpha$_2$ and gamma globulin values of 10.0 and 15.3 per cent, respectively. The total protein concentration of 7.0 gm./100 ml. is slightly below normal. All these changes are significant at the 0.1 per cent level of probability except for the increased beta globulin which is significant only at the 5 per cent level and the total protein which is not significant. The albumin was abnormally decreased in four cases with

TABLE IV

SERUM PROTEIN DISTRIBUTION AND CONCENTRATION IN PERSONS WITH NEOPLASTIC DISEASE

	No. of Persons	Albumin (%)	$Alpha_1$ Globulin (%)	$Alpha_2$ Globulin (%)	Beta Globulin (%)	Gamma Globulin (%)	Total Protein (gm./100 ml.)
Carcinoma and sarcoma:							
Metastatic	26	57.8	5.7	11.4	11.5	13.7	6.7
Standard deviation	..	9.8	2.4	3.8	3.3	3.2	0.6
Significance (p)	..	0.001	0.001	0.001	0.001	0.001	0.001
Non-metastatic	31	64.5	3.7	7.9	10.3	13.6	7.1
Hodgkin's disease	13	61.5	4.1	9.5	10.9	14.1	7.0
Standard deviation	..	2.0	1.3	2.1	1.6	1.5	0.5
Significance (p)	..	0.001	0.001	0.001	0.001	0.01	None
Benign tumors	33	68.0	3.0	7.5	8.7	12.8	7.4

values ranging down to 49 per cent and $alpha_2$ globulin was abnormally increased in four cases with values greater than the mean normal value found in all but one instance. Beta globulin and gamma globulin were elevated in one case each, and the $alpha_2$ globulin and total protein concentration were decreased in one case each.

Of a total of twenty-one cases of either cystitis, urethritis, epididymitis or prostatitis, five showed an increased $alpha_2$ globulin, four a decreased albumin, three an increased $alpha_1$ globulin, one a low gamma globulin and one a low total protein concentration. Sixteen of the twenty-one patients had greater than average concentrations of $alpha_2$ globulin.

Of nine patients with infectious mononucleosis, three showed a decreased concentration of albumin, two an increased $alpha_2$ globulin and one an increased gamma globulin concentration.

In thirty-eight miscellaneous infectious diseases $alpha_2$ globulin was abnormally elevated in thirteen cases, albumin was decreased in six and elevated in one, gamma globulin was increased in three cases and total protein concentration was elevated in one case and decreased in one case. Two cases of acute poliomyelitis, two cases of rubella, one case of coccidiomycosis and three cases of syphilis (late cases and probably inactive) all showed normal patterns. One case each of scarlet fever, chicken pox and Q fever showed elevated $alpha_2$ globulin levels, as did two of three cases of atypical pneumonia. One patient with acute rheumatic fever had a lowered albumin (51 per cent); one of three patients with malaria had an elevated gamma globulin, and four patients with extrapulmonary tuberculosis included one with a low albumin and one each with elevated gamma and $alpha_2$ globulin concentrations. In one case of schistosomiasis there was a low concentration of albumin (58 per cent) with an elevated total protein concentration (11.5 gm./100 ml.). Two patients with lymphogranuloma venereum had elevated gamma globulin concentrations (17.2 and 18 per cent).

Neoplasms. Of a group of fifty-seven cases of carcinoma and sarcoma, twenty-six were metastatic, including ten cases that resulted in death, and thirty-one without known metastases; these two groups were considered separately. The mean values for each group are shown in Table IV.

In the group with metastases there is a marked lowering of the mean albumin concentration to 57.8 per cent accompanied with an increase in the concentration of all globulin fractions, particularly the $alpha_2$ globulin (11.4 per cent), and a fall in the total protein concentration to 6.7 gm./100 ml. All these changes are statistically highly significant ($p = <0.001$). In ten cases albumin concentration was below the normal range and in every case but one the albumin concentration was below the average normal value. In four cases $alpha_2$ globulin and in three cases $alpha_1$ globulin were above normal. Total protein concentration was below normal in only two cases. The deviations from normal of the mean values

for this group of patients are also similar to those found by Seibert et al.[42] in a group of twenty-three patients with carcinoma.

The mean values for the group of thirty-one cases without known metastases show the same trend as those with metastases but to a much smaller extent. Most of the rise in the globulin fractions may be accounted for by the fall in albumin concentration. In this group five patients exhibited a lowered concentration of albumin (these included two deaths in this group and one case of co-existent cirrhosis of the liver) and twenty-three of the thirty-one cases exhibited albumin concentrations below the mean normal value. Gamma globulin concentration was slightly elevated in four cases, $alpha_2$ globulin was slightly elevated in two and decreased in two cases, $alpha_1$ globulin was slightly increased in two cases and total protein concentration was decreased in one case.

It can be concluded that although there is a definite tendency toward a lowering of the albumin concentration and a rise in the $alpha_2$ globulin concentration in cases of carcinoma and sarcoma, this tendency is largely dependent upon the presence of widespread disease, as in the metastatic group, and probably does not represent a specific abnormality due to the presence of malignancy.

The mean values for the protein distribution in thirteen cases of Hodgkin's disease (including three deaths) are also shown in Table IV. The protein pattern in this group of patients is similar to that found in carcinoma and sarcoma and the differences from normal of the albumin, $alpha_1$, $alpha_2$ and beta globulin values are also all highly significant statistically ($p = <0.001$), although the increase in gamma globulin concentration is significant only at the 1 per cent level and the small drop in total protein concentration is not significant. The albumin concentration was below normal in four cases and below the average normal value in all but one case. The $alpha_2$ globulin was elevated in four cases and the beta globulin in two; the level of each of these fractions was above the normal mean value in all but two cases and one case, respectively. Gamma globulin was elevated in one case.

Among five cases of leukemia the only markedly abnormal observation was an abnormal protein with the mobility of a gamma globulin in serum from a patient with leukemia of undetermined type. Two patients with acute monocytic leukemia had a normal protein distribution, one patient with chronic granulocytic leukemia had a slightly low albumin of 58 per cent and a patient with acute granulocytic leukemia had a low total protein concentration of 5.3 gm./100 ml. One patient with polycythemia vera had a slightly lowered albumin of 59 per cent with a total protein concentration of 9 gm./100 ml. One case of multiple myeloma showed a typical band of abnormal protein in the gamma region which accounted for over half of the elevated total protein concentration.

Thirty-three cases of various types of benign tumors showed an essentially normal distribution of protein. (Table IV.) The albumin was slightly lowered (59 per cent) in one case of neurofibromatosis. $Alpha_1$ globulin was elevated in two cases, $alpha_2$ globulin in two cases, gamma globulin in two cases and total protein concentration in one case; all of these abnormalities were slight. Two patients with chromophobe adenomas of the pituitary had normal protein distributions.

Although these results do not suggest the presence of a specific abnormality in protein distribution due to malignant disease, it is worthy of note that in a total of seventy-six cases of malignancy the albumin concentration was below the mean normal value in sixty-six instances and in the thirty-three cases of benign tumor the albumin concentration was below the normal range in only one instance. Thus the electrophoretic pattern may sometimes be of value in the diagnosis of malignant disease, since an albumin concentration above the mean normal value of 69 per cent is unlikely in cases with malignancy while a value below the range of normal is unlikely in cases with benign neoplasms or no disease.

Cardiovascular Disease. Fifty-eight cases with arteriosclerosis as the principal discharge diagnosis were divided into two groups: a group of thirty-nine patients in which the diagnosis was considered proved (patients with cerebrovascular accidents, myocardial infarctions, angina pectoris and arteriosclerosis obliterans) and a group of nineteen patients with a clinical diagnosis of arteriosclerosis only. The mean values for these two groups are given in Table V, and it can be seen that they do not differ appreciably from each other. The group of proved cases has a lowered albumin concentration of 61.1 per cent accompanied with elevations in all the globulin fractions and a slightly decreased

TABLE V

SERUM PROTEIN DISTRIBUTION AND CONCENTRATION IN PERSONS WITH CARDIOVASCULAR DISEASE

	No. of Persons	Albumin (%)	Alpha$_1$ Globulin (%)	Alpha$_2$ Globulin (%)	Beta Globulin (%)	Gamma Globulin (%)	Total Protein (gm./100 ml.)
Arteriosclerosis proved	39	61.1	3.9	9.7	11.0	14.4	7.0
Standard deviation	..	7.6	1.4	2.5	1.8	4.0	0.5
Significance (p)	..	0.001	0.001	0.001	0.001	0.001	0.01
Arteriosclerosis diagnosed	19	61.7	4.3	9.5	11.5	13.1	6.5
Rheumatic heart disease	18	60.9	4.3	8.4	10.5	16.0	7.2
Standard deviation	..	6.4	1.3	2.2	2.4	2.8	0.7
Significance (p)	..	0.001	0.001	0.01	0.01	0.001	None

total protein concentration. All these changes are statistically highly significant ($p = <0.001$) except for the fall in total protein concentration, which is significant at the 1 per cent level. It is of interest that the beta globulin fraction, which is associated with the majority of the plasma lipid, shows no greater rise than the other globulin fractions. In seventeen of these thirty-nine cases there was an abnormally low albumin concentration, with values as low as 43 per cent, and all but six had values below the average normal value. Four patients had an elevated alpha$_2$ globulin, one had a low gamma globulin, and none had an abnormally elevated beta globulin fraction. The nineteen subjects with a clinical diagnosis of arteriosclerosis show a similar picture with seven abnormally low albumin values ranging down to 44 per cent, four elevated alpha$_2$ globulin values, two elevated beta globulin values and five cases in which there was a decreased concentration of total protein.

The decrease in albumin concentration found in these patients, most of whom were seriously ill and eight of whom died, is similar to that observed by Leinwand and Moore[43] with moving boundary electrophoresis in a group of patients with arteriosclerosis. It is in contrast to the small decrease in albumin concentration found in a group restricted to patients with a history of myocardial infarction, most of whom were not critically ill at the time of serum protein analysis.[44] These results suggest that the fall in albumin concentration in the group of patients reported herein may be an indication of the degree of illness of these patients rather than a specific indication of arteriosclerosis itself.

In seventeen cases of hypertension beta globulin was elevated in three patients, albumin concentration was 58 per cent in one case and gamma globulin was elevated to 22 per cent in another case.

The mean values for concentration of protein fractions in eighteen cases of rheumatic heart disease are shown in Table v. The albumin is lowered to 60.9 per cent and there is a rise in all the globulin fractions which is appreciable only in the case of the gamma globulin with a mean value of 16.0 per cent; the total protein concentration is normal. The fall in albumin concentration and the rise in both gamma globulin and alpha$_1$ globulin concentration are highly significant statistically ($p = <0.001$). The increases in alpha$_2$ and beta globulin concentration are significant at the 1 per cent level, while total protein concentration is not significantly different from normal. The albumin concentration was below normal in five cases (lowest value 42 per cent) and gamma globulin concentration above normal in six cases. In addition, beta globulin was abnormally elevated in two cases and alpha$_2$ globulin in one case.

In nine cases of thrombophlebitis alpha$_2$ globulin, with an average concentration of 9.3 per cent, was elevated above the mean normal value in all cases but one and elevated above the normal range in three cases. In addition, albumin concentration was decreased to 53 per cent in one instance and beta globulin increased in one instance.

Respiratory Diseases. The average values for concentration of protein fractions in thirteen cases of bronchial asthma are shown in Table VI.

TABLE VI

SERUM PROTEIN DISTRIBUTION AND CONCENTRATION IN PERSONS WITH BRONCHIAL ASTHMA AND INFECTIOUS HEPATITIS

	No. of Persons	Albumin (%)	$Alpha_1$ Globulin (%)	$Alpha_2$ Globulin (%)	Beta Globulin (%)	Gamma Globulin (%)	Total Protein (gm./100 ml.)
Bronchial asthma	13	63.3	3.5	9.0	10.0	14.3	7.5
Standard deviation	..	4.3	1.1	1.9	1.1	2.6	0.7
Significance (p)	..	0.001	0.05	0.001	0.05	0.01	None
Infectious hepatitis	24	67.4	2.7	6.6	9.1	14.2	7.5

The decrease in albumin concentration to 63.3 per cent is accompanied with an increase in the concentration of all globulin fractions, particularly the $alpha_2$ and gamma globulins. The changes in albumin and $alpha_2$ globulin are significant at the 0.1 per cent level, the gamma globulin at the 1 per cent level, and the $alpha_1$ and beta globulins at the 5 per cent level, while the total protein concentration is not significantly different from normal. Albumin concentration was abnormally low in three cases, $alpha_2$ globulin was abnormally high in three cases, gamma globulin was elevated in two cases and $alpha_1$ globulin was above normal in one case.

In the nine cases of emphysema and fibrosis an $alpha_2$ globulin concentration of 3.8 per cent was encountered once.

Five cases of pneumothorax had normal patterns except for one case with an elevated $alpha_1$ globulin and one with an elevated $alpha_2$ globulin.

Digestive System Diseases. Only three cases of cirrhosis of the liver were encountered. All three showed the typical pattern of decreased albumin concentration (40 per cent, 49 per cent and 50 per cent) and two showed markedly elevated gamma globulins (both 31 per cent). One patient had, in addition, an elevated beta globulin and only one of the three had a low total protein concentration (4.5 gm. per cent). The abnormal paper electrophoretic patterns of these three cases were easily discernible on inspection of the strip without scanning.

A group of twenty-four cases of infectious hepatitis showed a small proportion of abnormalities, probably because many of the cases represented patients in late stages of convalescence who had been transferred from other hospitals. Five cases showed a typical increase in gamma globulin concentration to 17 to 19 per cent while only one had a lowered albumin (53 per cent). The total protein concentration was elevated to 9.0 gm./100 ml. in one case and was low in none. The mean concentrations of the protein fractions in these cases are shown in Table VI.

Serum from seventeen patients with duodenal ulcers showed an elevated $alpha_2$ globulin in four cases, a lowered albumin in two cases, and an elevated $alpha_1$, beta and gamma globulin fraction and total protein concentration in one case each.

Of the nine cases with enteritis or colitis an elevated $alpha_2$ globulin was found in three cases and a decreased albumin in three cases. Elevated $alpha_1$ and gamma globulins were found in one case each. One case of ulcerative colitis with an albumin level of 50 per cent was included among the three with depressed albumin levels.

Genitourinary Diseases. Five cases of glomerulonephritis were studied. Two of these had elevated levels of beta globulin (15 per cent and 17 per cent) and in all cases the beta globulin was above the mean normal value. The $alpha_2$ globulin was elevated to 12 per cent in one case and the albumin was decreased to 53 per cent in one case. The total protein concentration was below normal in two cases.

Among fifteen cases of renal and ureteral calculi $alpha_2$ globulin was increased in three instances and albumin concentration decreased in three instances. Beta globulin was increased in one case and total protein concentration was decreased in one case.

In twenty-three cases of benign prostatic hypertrophy albumin concentration was de-

TABLE VII

SERUM PROTEIN DISTRIBUTION AND CONCENTRATION IN PERSONS WITH DISEASES OF THE LOCOMOTOR SYSTEM

	No. of Persons	Albumin (%)	Alpha$_1$ Globulin (%)	Alpha$_2$ Globulin (%)	Beta Globulin (%)	Gamma Globulin (%)	Total Protein (gm./100 ml.)
Rheumatoid arthritis	27	64.3	3.1	8.3	10.1	14.3	7.7
Standard deviation	..	5.3	0.8	1.9	2.2	3.6	0.7
Significance (p)	..	0.001	None	0.001	0.01	0.001	0.01
Osteoarthritis	12	68.3	3.4	7.4	9.2	11.5	8.0
Standard deviation	..	3.0	1.1	0.9	1.1	2.0	1.0
Significance (p)	..	None	None	None	None	None	0.001
Fractures	34	65.7	3.4	8.6	10.0	12.3	7.2
Standard deviation	..	4.4	1.1	1.8	1.9	2.7	0.6
Significance (p)	..	0.001	0.01	0.001	0.01	None	None
Herniated nucleus pulposus	34	67.9	2.6	7.2	9.2	13.2	7.3

creased in four cases, with values as low as 51 per cent, and total protein concentration was decreased in one case. Alpha$_1$ globulin and beta globulin were each elevated in one case and alpha$_2$ globulin in two cases.

Locomotor System Diseases. The mean values for distribution of protein fractions in a group of twenty-seven patients with rheumatoid arthritis are shown in Table VII. A rise in the mean gamma globulin concentration to 14.3 per cent is accompanied with a smaller rise in the other globulin fractions and a small drop in the concentration of albumin to 64.3 per cent; the total protein concentration of 7.7 gm./100 ml. is above normal. The rise in gamma and alpha$_2$ globulins and the fall in albumin are highly significant at the 0.1 per cent level and the rise in beta globulin and total protein concentration are significant at the 1 per cent level; the change in alpha$_1$ globulin is not significant. In five cases gamma globulin was elevated above the normal range with values up to 20 per cent and in twenty of the twenty-seven cases it was greater than the mean normal value. The albumin concentration was slightly below normal in six cases and the alpha$_2$ and beta globulin fractions were increased in three and two cases, respectively. In two cases total protein concentration was above normal.

In marked contrast to the findings in the series of patients with rheumatoid arthritis a group of twelve patients with osteoarthritis presented no abnormality whatever in protein distribution except for one case with an elevated alpha$_1$ globulin. In one case there was an elevated total protein concentration and in another this was decreased. The mean values for this group are also shown in Table VII. None of the values vary greatly from the normal except for the total protein concentration, which is significantly increased largely as a result of one very high value.

The mean values for a group of thirty-four patients with herniated nucleus pulposus are shown in Table VII; there is no appreciable variation from the normal levels. The individual analyses were all normal except for three instances of slightly elevated gamma globulin and one instance of elevated beta globulin concentration. The total protein concentration was elevated in one case.

The mean values for a group of thirty-four patients with fractures as a result of trauma are shown in Table VII. The average albumin concentration of 65.7 per cent is slightly below normal and the alpha$_2$ globulin concentration of 8.6 per cent is above normal; both of these changes are, however, highly significant ($p = <0.001$). A smaller increase is seen in the alpha$_1$ and beta globulins ($p = <0.01$), while the gamma globulin and total protein concentrations are not significantly different from normal. In the individual analyses eight of the alpha$_2$ determinations were above the normal range of variation. The albumin concentration was slightly decreased in three cases and the beta globulin increased in one.

TABLE VIII

SERUM PROTEIN DISTRIBUTION AND CONCENTRATION IN PERSONS WITH MISCELLANEOUS DISEASES

	No. of Persons	Albumin (%)	$Alpha_1$ Globulin (%)	$Alpha_2$ Globulin (%)	Beta Globulin (%)	Gamma Globulin (%)	Total Protein (gm./100 ml.)
Sarcoidosis	8	64.6	2.7	7.9	10.2	14.6	7.3
Standard deviation	..	3.9	0.8	0.8	1.6	3.5	0.8
Significance (p)	..	0.01	None	None	None	0.01	None
Ocular diseases	32	68.5	2.8	7.3	9.0	12.5	7.3
Deafness	32	69.6	2.8	7.3	9.2	11.0	7.2
Miscellaneous congenital diseases	41	68.6	3.1	7.6	9.2	11.5	7.2

In twenty-nine patients hospitalized with conditions resulting from recent trauma, largely sprains and dislocations, there were no abnormalities except for one lowered albumin (57 per cent), one increased $alpha_2$ globulin (11 per cent) and one increased beta globulin (13 per cent) concentration.

In a group of sixty-five cases of disorders of the locomotor system as a remote result of disease or trauma there were two patients with an elevated albumin concentration, three with a decreased albumin concentration, four with an elevated $alpha_2$ globulin concentration and one with a decreased $alpha_2$ globulin concentration.

Dermatologic Conditions. In eleven cases of neurodermatitis disseminata there were two persons each with elevated gamma and $alpha_2$ globulin levels and one patient with an elevated beta globulin. Among ten cases of widespread infections of the skin albumin concentration was decreased to 58 per cent in one patient and beta and gamma globulins were each increased in one patient. In a group of twenty-four other patients with various dermatologic diseases two patients with psoriasis, three with atopic dermatitis and two with seborrheic dermatitis had normal electrophoretic patterns. Of five patients with dermatitis venenata one had an increased gamma globulin concentration of 19 per cent and one a slightly decreased albumin concentration. One patient with erythema multiforme had a gamma globulin level of 20 per cent.

Other Diseases. The only remarkable finding in a group of six cases of various types of anemia was a serum gamma globulin level of 21 per cent in a patient with sickle cell disease. One patient with pernicious anemia had a normal protein distribution and three patients with hypochromic anemia were normal except for one lowered albumin (58 per cent) in a patient who also had severe arteriosclerosis.

Twelve cases of diabetes showed an essentially normal pattern except for a tendency toward elevation of the beta globulin fraction. This fraction was above the normal range in only two cases but the mean value of 10.9 per cent is significantly ($p = <0.01$) greater than normal. One case exhibited an albumin concentration of 59 per cent.

Normal electrophoretic patterns were found in three cases of hypothyroidism, two cases of hyperthyroidism, two cases of acromegaly and one case of congenital hyperplasia of the adrenals.

The mean values obtained in a group of eight cases of sarcoidosis are shown in Table VIII. The small but significant ($p = <0.01$) rise in gamma globulin and fall in albumin accompanied with no appreciable change in the $alpha_1$, $alpha_2$ and beta fractions are similar to the changes found by Seibert et al.[42] in a group of eleven cases, but of smaller magnitude, possibly because many of the cases reported herein had very localized disease. However, in the absence of a significant rise in the concentration of total protein in this group of cases, these data do not support the conclusion of these authors that analysis of serum proteins may be of value in the differential diagnosis of sarcoidosis and tuberculosis, with the possible exception of cases of markedly elevated $alpha_2$ globulin. The individual analyses in this group were all within normal limits except for two instances of an elevated gamma globulin concentration. All but one of the patients had

gamma globulin levels above the average normal values and all but one had albumin concentrations below the normal average.

The mean values for protein distribution in a group of thirty-two patients with miscellaneous diseases of the eye are shown in Table VIII. None of the values differ appreciably from normal. All the individual values in this group were within normal limits except for one case with a lowered albumin concentration. Six cases of chorioretinitis included in this group were all normal.

This group includes thirty-two cases of deafness which were not included in the group used to establish normal limits because of the presence of trauma or otosclerosis, which might have led to alterations in protein patterns. The mean values shown in Table VIII for this group are essentially normal and indicate that no such alteration exists. The individual analyses were all normal except for one case with an albumin concentration of 59 per cent.

The forty-one cases of miscellaneous congenital conditions represent a variety of congenital anatomic defects, most of them minor. The mean values for protein distribution and total protein concentration for this group are given in Table VIII and do not differ appreciably from the normal values. Individual analyses were normal except for two elevated beta globulin values, one each of elevated alpha$_1$ and gamma globulin values, and one increased and one decreased total protein level. Five cases of coarctation of the aorta included in this group had normal protein distributions.

The normal mean values and the low incidence of abnormal patterns in these last three groups are of interest in that they suggest abnormalities found in other disease groups, even with a small number of cases, are indeed abnormalities and do not represent technical errors or random variation.

Miscellaneous Diseases. Fifty-nine analyses were carried out on serum from patients with diseases of which only one to three representative specimens were obtained, and only the results of particular interest will be reported here. Two cases of chondromalacia and one case each of Huntington's chorea, multiple sclerosis, constitutional hyperbilirubinemia, acute cholecystitis, acute polyneuritis, pancreatitis, alcoholic neuropathy, polyostotic fibrous dysplasia, orthostatic albuminuria and thrombocytopenic purpura all exhibited normal protein patterns. In three cases of hydronephrosis there was one instance of a lowered albumin (46 per cent) and two increased beta globulin levels. One of two cases of lupus erythromatosus had a lowered albumin concentration (50 per cent), and one patient with uremia had markedly decreased albumin (35 per cent) and total protein (5.1 gm./100 ml.) concentrations with an elevated gamma globulin level (39 per cent). One case of xanthomatosis had an elevated total protein concentration with a 17 per cent concentration of gamma globulin. One patient with Osgood-Schlatter's disease had an elevated beta globulin (14.9 per cent), one with gout had a slightly elevated alpha$_2$ globulin (11 per cent), one with serum sickness had a low concentration of both albumin (49 per cent) and total protein (5.8 gm./100 ml.), and one with eosinophilic pneumonitis had a slightly elevated alpha$_1$ globulin (5.7 per cent). One patient with hypogammaglobulinemia had a gamma globulin level of 2.8 per cent with a normal concentration of the other fractions, and a case of nephrosis exhibited a typical pattern of markedly lowered albumin and gamma globulin (both 11 per cent with a total protein concentration of 2.9 gm./100 ml.) and elevated alpha$_2$ globulin (54 per cent).

Obstetric Patients and Newborn Infants. Aside from blood specimens obtained from routine hospital admissions, analyses were carried out on a group of 325 specimens from obstetric patients, most of which were taken three days postpartum, and on a group of forty-one specimens from cord blood of newborn infants. A summary of the data obtained from these patients is given in Table IX.

The mean values for concentrations of electrophoretically separated protein fractions in the group of cord bloods are almost identical with normal adult values for albumin and alpha$_2$ globulin, while the beta globulin (7.6 per cent) and alpha$_1$ globulin (2.5 per cent) are below the mean adult values and the gamma globulin of 13.5 per cent is above the adult value. The total protein concentration of 5.7 gm./100 ml. is considerably below the adult figure so that absolute concentrations of all fractions, except possibly the gamma globulin, are lower than in the adult. It is likely that the comparatively low values for the alpha$_1$ and beta globulins may be accounted for at least partly by the low concentration of lipoproteins in the newborn infant.[45] The differences from normal for the beta globulin, gamma globulin and total protein

TABLE IX

SERUM PROTEIN DISTRIBUTION AND CONCENTRATION IN POSTPARTUM WOMEN AND NEWBORN INFANTS

	No. of Persons	Albumin (%)	Alpha$_1$ Globulin (%)	Alpha$_2$ Globulin (%)	Beta Globulin (%)	Gamma Globulin (%)	Total Protein (gm./100 ml.)
Postpartum women	325	57.6	5.0	11.0	13.7	12.7	6.6
Standard deviation	...	6.5	1.1	2.1	2.4	2.9	1.5
Significance (p)	...	0.001	0.001	0.001	0.001	0.01	0.001
Newborn infants	41	69.2	2.5	7.2	7.6	13.5	5.7
Standard deviation	...	3.1	0.7	0.4	1.7	2.8	0.8
Significance (p)	...	None	0.01	None	0.001	0.001	0.001

values are highly significant statistically ($p = <0.001$); the decrease in alpha$_1$ globulin is significant at the 1 per cent level, while the albumin and alpha$_2$ globulins are not significantly different from normal. It is of some interest that the standard deviations for each protein fraction are smaller than the comparable standard deviations in the adult group for all fractions except the gamma globulin. This indicates that the newborn infant has a more constant serum protein composition than the adult in all fractions except the gamma globulin, which is supposed to be composed partly of gamma globulins from the maternal blood and expected to be more variable.

The general pattern of protein distribution in the group of postpartum women is similar to that reported by Coryell et al.[19] in a study carried out on plasma with the moving boundary technic although the changes from normal in the present study are less marked than those reported by these authors. This is attributed to the differences in the groups studied and/or differences in technics used to measure protein concentration. The beta globulin concentration of 18.3 per cent found with the moving boundary method, for instance, may be compared with the 13.7 per cent found with the paper electrophoretic technic in which the lipid of beta lipoprotein makes little contribution to the measured protein concentration. The albumin concentration and the total protein concentration are considerably decreased to 57.6 per cent and 6.6 gm. per 100 ml., respectively, while the alpha$_1$, alpha$_2$ and beta globulin fractions are elevated to 5.0, 11.0 and 13.7 per cent, respectively. Although the gamma globulin concentration of 12.7 per cent is not very different from the normal value in terms of per cent, the lowered total protein concentration indicates that absolute concentration of gamma globulin is below normal. All these changes are highly significant statistically ($p = <0.001$) except for the gamma globulin which is significant at the 1 per cent level. The standard deviations for all fractions are greater than those found in the normal group, indicating that the degree of variation in serum protein distribution in this group of patients is greater than normal.

Only a small number of specimens from patients with abnormal pregnancies were obtained. In a group of ten patients with pre-eclampsia of varying degrees of severity only one grossly abnormal pattern was found in which the albumin concentration was decreased to 44 per cent and the beta globulin concentration increased to 24 per cent with a total protein concentration of 4.5 gm./100 ml. One diabetic patient had a normal pattern and one Rh-negative patient with a high antibody level on cortisone therapy had an albumin concentration of 51 per cent and a beta globulin of 18 per cent.

Distribution of Abnormalities by Disease. A summary of the abnormal findings in serum protein distribution and concentration arranged by disease groups is given in Table x. The results obtained from newborn infants, postpartum women and the normal group are not included. This table represents the incidence and distribution of serum protein abnormalities in a hospital population which is predominantly male and in the younger age group. It does not include patients with psychiatric disorders.

The most common abnormality in protein distribution noted in this series of cases was a

TABLE X

SIGNIFICANCE AND INCIDENCE OF ABNORMALITIES IN SERUM PROTEIN DISTRIBUTION AND CONCENTRATION IN A GROUP OF 965 PATIENTS

	No. of Persons	Albumin		Alpha$_1$ Globulin		Alpha$_2$ Globulin		Beta Globulin		Gamma Globulin		Total Protein	
		High	Low	High	Low	High	Low	High	Low	High	Low	High	Low
Infections:													
Pulmonary tuberculosis	22	..	10	..	..	3	..	2	..	5	..	..	3
Other pulmonary infections	47	..	6	1	..	12	..	1	..	1	..	..	2
Other infections	118	1	21	7	..	24	2	1	1	6	1	1	3
Neoplasms:													
Malignant	76	..	23	5	..	10	2	2	..	6	..	2	4
Benign	33	..	1	2	..	2	..	..	..	2	..	1	..
Cardiovascular diseases:													
Arteriosclerosis	58	..	23	..	..	8	..	2	..	1	1	..	5
Rheumatic heart disease	18	..	5	..	..	1	..	2	..	6	..	..	..
Other	26	..	2	..	..	3	..	4	..	1	..	..	..
Respiratory diseases:													
Bronchial asthma	13	..	3	1	..	3	..	..	..	2	..	..	..
Other	14	..	2	1	..	1	1	..	..	..	..	..	..
Digestive system diseases:													
Hepatitis	24	..	1	..	..	...	..	..	..	5	..	1	..
Cirrhosis	3	..	3	..	..	...	..	1	..	2	..	..	1
Ulcers, enteritis, and the like	26	..	5	2	..	7	..	1	..	2	..	1	..
Genitourinary diseases:													
Calculi, glomerulonephritis, benign prostatic hypertrophy	41	..	8	1	..	6	..	4	..	..	..	..	5
Locomotor diseases:													
Rheumatoid arthritis	27	..	6	..	..	3	..	2	..	5	..	2	..
Osteoarthritis	12	..	...	1	..	...	..	..	..	..	..	1	1
Other mechanical and traumatic conditions	162	2	7	..	1	13	1	3	..	3	..	1	..
Dermatologic conditions	45	..	3	1	..	4	..	2	..	6	..	..	..
Other diseases:													
Endocrine diseases	20	..	1	..	..	...	..	2	..	..	..	..	..
Ocular diseases	32	..	1	..	..	...	..	..	..	..	..	..	..
Deafness	32	..	1	..	..	...	..	..	..	..	..	..	..
Sarcoidosis	8	..	...	..	..	...	..	..	..	2	..	..	..
Miscellaneous congenital diseases	41	..	...	1	..	1	..	2	..	1	..	1	1
Miscellaneous diseases	67	..	11	3	1	3	..	4	..	4	2	3	2
Total	965	3	143	26	2	104	6	35	1	60	4	14	27

decrease in albumin concentration, which was observed in 143 of the 965 cases studied. A second common abnormality was an elevation of the alpha$_2$ globulin, which occurred in 104 cases, followed by an increase in gamma globulin in sixty cases. Increased levels of beta globulin and alpha$_1$ globulin occurred rarely, with only thirty-five and twenty-six instances, respectively. Elevated concentrations of albumin and lowered concentrations of individual globulin fractions were unusual and probably represented random variation in most instances. Abnormalities in the concentration of total protein were much less common than abnormalities in protein distribution, occurring in only forty-one instances.

The 321 cases of malignant disease, arteriosclerotic cardiovascular disease and infectious disease account for the major portion (eighty-three) of lowered albumin levels, with tuberculosis and arteriosclerosis showing nearly a 50 per cent incidence of this abnormality. Patients with cirrhosis of the liver, nephrosis and multiple myeloma, although rare in this series, all had lowered albumin levels, and the cases of rheumatoid arthritis and rheumatic heart disease made a significant contribution to this group.

The patients with an elevated alpha$_2$ globulin were fairly widely distributed but in general followed the expected pattern of increased frequency in diseases involving tissue inflammation and destruction. By far the most common cause of this abnormality was infectious disease, the 187 cases of which accounted for thirty-nine instances. It was also frequently found in association with malignancy, ulcers and inflammation of the intestinal system, fractures and arteriosclerotic disease, although much less frequent in the last condition than a decreased albumin concentration. The one case of nephrosis had a markedly elevated alpha$_2$ globulin.

An elevation in gamma globulin was most fre-

quently associated with long-standing infections in which one might expect an elevated antibody level (but was much less common in this group than either an elevated $alpha_2$ globulin or lowered albumin concentration) and was also frequently observed in rheumatic heart disease, infectious hepatitis, cirrhosis, rheumatoid arthritis, dermatologic conditions and malignancy. A lowered gamma globulin appeared to be of significance only in the single case of hypogammaglobulinemia.

Increased levels of beta and $alpha_1$ globulin were quite widely scattered and appeared to be of little diagnostic significance. It is of some interest that the beta globulin fraction was elevated in only two of the fifty-eight cases of arteriosclerosis. Changes in concentration of total protein were also widely scattered and were of diagnostic significance in only a comparatively few instances, such as multiple myeloma and nephrosis.

Detection of Abnormalities by Inspection of the Stained Paper Strips. It would be of value for some clinical purposes if abnormalities in the concentration of protein fractions could be detected by simple inspection of stained strips without resorting to scanning or elution of dye.

To test the accuracy of such a procedure the numbered strips from this series of determinations (not including the obstetric cases) were examined by an observer with no knowledge of the diagnoses or results of scanning for each strip. The results of his observations were then compared with the results obtained by scanning the same strip. Such a comparison indicated that an albumin concentration which appeared to be abnormally low on inspection was actually below normal in only 50 per cent of the cases although an apparently elevated gamma globulin concentration was actually elevated in 90 per cent of the cases. Conversely, about two-thirds of the actually lowered albumin fractions but only one-quarter of the actually elevated gamma globulin fractions were detected by inspection. The results with other globulin fractions were even less satisfactory. It was concluded that while grossly abnormal patterns could be correctly identified by inspection, such a procedure was inaccurate in detecting moderate deviations from normal and provided considerably less information than the quantitative procedure.

Lipoprotein Patterns. Although no quantitative evaluation of serum lipoprotein distribution was attempted in this study, a qualitative estimate of gross abnormalities of lipoprotein concentration and distribution was attempted. Strips on which 0.02 ml. of serum had been separated and stained with the lipid dye oil red O[46] were coded by number and examined by an observer who had no knowledge of the diagnosis relating to each specimen. Apparent abnormalities, usually elevated beta lipoprotein or decreased alpha lipoprotein, were noted in this way in about 10 per cent of the patterns in both the control series and the group of ill patients. Markedly increased beta lipoprotein concentrations were evident in three cases of glomerulonephritis which also exhibited an elevated beta globulin level in the protein pattern, and in one case of xanthomatosis. In addition, a clearly split beta lipoprotein peak was observed in one pattern obtained from a presumably normal pregnant woman. In general, the qualitative analysis of serum proteins by this method appeared to provide little information and to be of value only in a few cases.

COMMENTS

The information of significance in the diagnosis and treatment of disease obtained from electrophoretic analysis of serum may be divided into two categories. First, information about the state of disturbed physiology reflected in abnormalities in the serum protein pattern and which may be of value in the estimation of prognosis, severity or type of disease. Second, more specific but also more empiric diagnostic information as to the particular disease or group of diseases likely to be associated with a certain type of abnormal protein distribution.

The type of abnormality included in the first group is by far the more common. The most frequent abnormality of this kind is a decrease in albumin concentration noted in 143 cases in the present series, not including the postpartum women and which occurs frequently in association with disease of a generalized and fairly severe nature. The cause of this abnormality is unknown except for those cases in which the ability of the liver to synthesize albumin has been impaired or in which there is a considerable loss of albumin in the urine. The rise in $alpha_2$ globulin which is often observed in association with inflammation and tissue destruction appears to be largely due to an increase in the concentration of glycoproteins,[42] although little is known about the mechanism or physiologic

significance of this increase. Abnormalities in these two serum protein fractions may serve as indications of the severity of disease in the same way as the erythrocyte sedimentation rate and the body temperature. A correlation of these abnormalities with the sedimentation rate has been found by Shedlovsky and Scudder.[47] It is noteworthy that a fall in albumin concentration accompanied with a rise in alpha globulin level is not always evident from the results of a routine salt fractionation determination of serum albumin since most of the alpha globulins are left in solution with this technic and measured along with the albumin fraction.

The gamma globulin fraction is most frequently elevated in chronic infections, connective tissue diseases and certain liver diseases. Although the elevation of this fraction may be ascribed in part to the formation of antibodies in infections and possibly in some other conditions, its significance remains controversial in the latter group. The beta globulin fraction contains the beta lipoprotein(s) and an increase in this fraction may indicate an increased lipoprotein concentration. Some of the conditions with a greatly elevated concentration of serum lipids, such as nephrosis, have the majority of lipid in a lipoprotein with the mobility of an $alpha_2$ globulin. Whether this represents an entirely different lipoprotein from the normal beta lipoprotein, or is merely a beta lipoprotein of slightly altered composition which moves with a greater mobility than normal, is at present undetermined. Although abnormalities of this kind are relatively non-specific, they may nevertheless be of considerable value as indicators of the severity of disease and even of the diagnosis in such conditions as malignancy, arteriosclerosis, generalized or chronic infection, rheumatoid arthritis (versus osteoarthritis) and rheumatic fever.

The second group of abnormalities includes those protein distribution patterns of more specific value in diagnosis. There are only a few diseases in which the electrophoretic pattern alone has been virtually pathognomonic; the abnormal patterns found in multiple myeloma, the nephrotic syndrome and hypogammaglobulinemia are well known. There are a larger number of diseases including hepatitis, cirrhosis of the liver, tuberculosis, sarcoidosis, kala-azar and lymphogranuloma venereum in which the electrophoretic pattern, while not diagnostic by itself, is characteristically altered and may be highly suggestive of the diagnosis.

The determination of serum protein distribution by means of paper electrophoresis and dye binding represents a compromise between the simple but relatively imprecise and non-specific salt fractionation procedures used for routine albumin-globulin determinations, and the technically difficult but more precise determination of protein distribution in terms of refractive index increment by moving boundary electrophoresis. The salt fractionation technic has long been known to provide an incomplete separation between albumin and globulins and to be subject to errors and difficulties of interpretation because of such problems as adsorption of protein during filtration and variations in degree of precipitation, particularly with abnormal serum.[11] The moving boundary technic, on the other hand, while capable of giving excellent results is seriously limited in its application because of the time and equipment required for each analysis. It is further subject to difficulties in interpretation due to boundary anomalies, turbidity and interaction between proteins. The salt fractionation and moving boundary technics generally do not give results which are readily comparable to each other or to the paper electrophoresis technic. This is partly due to differences in the separations obtained with the various methods but would be noted even with identical separations because of the different units used to measure and express protein concentration in each case. The paper electrophoretic technic has the disadvantages of albumin tailing and use of a method for determination of protein concentration which is primarily dependent on the concentration of free amino groups in the denatured proteins[10] rather than the more familiar refractive index increment (which measures protein-bound lipid and carbohydrate as well), nitrogen or biuret value. It is for this reason that the normal values for albumin are higher when measured by the dye binding method than by the moving boundary technic, although they happen to agree closely with the values obtained by Gutman et al. with the salt fractionation method.[11] In addition, the deviation from linearity in the relationship between optical density and dye concentration on paper gives rise to the possibility of error in individual determinations if the albumin band is of markedly different shape from the albumin bands used to prepare the standard curve from which the correction factor is calculated. This has not been a source of serious error in our hands and can be avoided

by determination of dye binding by elution rather than direct scanning. When properly controlled, the paper electrophoretic technic provides quantitative results of sufficient accuracy for most purposes and has the advantages of simplicity and of providing a clear-cut separation of the five major electrophoretic fractions of serum.

SUMMARY

1. The results obtained by paper electrophoretic analysis of the serum protein distribution of 1,516 admissions to an army general hospital are described. The differences between results obtained by paper electrophoresis and by moving boundary electrophoresis, and the reasons for these differences are discussed briefly.

2. Infections (particularly tuberculosis), malignant neoplasms, arteriosclerosis, rheumatic heart disease, hepatitis, cirrhosis, rheumatoid arthritis and sarcoidosis were most frequently associated with abnormalities in serum protein distribution.

3. The most repeated abnormality in this series of cases was a decrease in the serum albumin concentration, followed by elevation of the alpha$_2$ globulin, gamma globulin, beta globulin and alpha$_1$ globulin. Increased levels of albumin and decreased levels of the serum globulins occurred rarely.

4. The serum protein distributions of 325 women and of forty-one newborn infants are described.

5. The results of paper electrophoretic analysis of the serum proteins may be pathognomonic for multiple myeloma, nephrosis and hypogammaglobulinemia, and useful in the diagnosis of hepatitis, cirrhosis, tuberculosis, sarcoidosis, kala-azar and lymphogranuloma venereum. In addition, information may be obtained regarding the state of disturbed physiology, the activity and, occasionally, the diagnosis in such conditions as rheumatoid arthritis, rheumatic fever, malignancy, sequelae of arteriosclerosis and infectious diseases.

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Seminar on Diseases of the Pancreas

Cystic Fibrosis of the Pancreas*

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CYSTIC fibrosis of the pancreas is a generalized, hereditary disease of children due to dysfunction of exocrine glands. It is characterized clinically in most instances by the combination of symptoms of pancreatic insufficiency and chronic pulmonary disease. However, gradation in or absence of involvement of the various organs or glandular systems affected (pancreas, lungs, liver, sweat glands) is characteristic of this disorder and leads to variations in the clinical picture according to the area predominantly involved.

Involvement of mucous glands (Table 1, Fig. 1D, 1E and 1F) throughout the body (intestinal, genitourinary tract and others) is occasionally found at autopsy but there are no recognized symptoms. Pathologically there is accumulation of what has been interpreted as abnormal mucus leading to dilatation of the secretory glands. Obstruction of the main passages of the pancreas, liver, lungs and small intestine, presumably by abnormal secretions, gives rise to the most striking clinical manifestations. No histologic changes are found in non-mucus-secreting glands (Table 1), although sweat has a markedly increased concentration of electrolytes and the parotid secretory rate is abnormal. Massive salt depletion through sweat in hot weather may lead to serious consequences, at times to death.

Cystic fibrosis of the pancreas is a relatively recently recognized condition. In 1936 Fanconi[1] described three cases and gave the disease its name. He recognized the association in these patients of the symptoms of pancreatic deficiency with chronic pulmonary disease. This paper, however, did not attract much attention and it was not until Andersen's[2] description in 1938 of forty-nine cases, gathered from the autopsy files of Babies Hospital in New York and from the world literature, that interest in the condition was aroused. In the same year two smaller series of patients were reported by Blackfan and May[3] in Boston and by Harper[4] in Australia. The pathologic lesions in the pancreas attracted the attention of the early investigators and gave the disease its name.

Prior to 1938 the diagnosis of cystic fibrosis had never been made from clinical evidence; the majority of patients died of bronchopneumonia, the basic disease having been unrecognized. In a smaller number the predominant symptoms of malabsorption led to the suspicion of celiac disease. In recent years, with the advent of effective antibiotic agents and with greater awareness of the disease and its clinical variations, an increasingly large number of such patients is seen in pediatric centers in this country and abroad.

Fibrocystic disease of the pancreas is not a rare disorder. In the seventeen years from 1939 through May 1956, 397 cases have been seen at Babies Hospital. Other clinics in this country and abroad have each observed several hundred cases. A review of the necropsy files in this and other institutions[2,5] has revealed that 2 to 4 per cent of patients subjected to autopsy in the pediatric age group suffered from this disease. The incidence in the general population has been estimated by Andersen[6] as being 1.9 per 1,000 live births based on an autopsy rate of 3 per cent in relation to the vital statistics of New York City. Since this condition is familial and thought generally to be transmitted as a recessive trait,[5–8] as many as 2 to 18 per cent of the population may harbor the specific gene.[7]

Cystic fibrosis has striking racial predilections. It occurs equally in all groups of the white race and has been recognized in widely separated countries. It is rare in Negroes and has never

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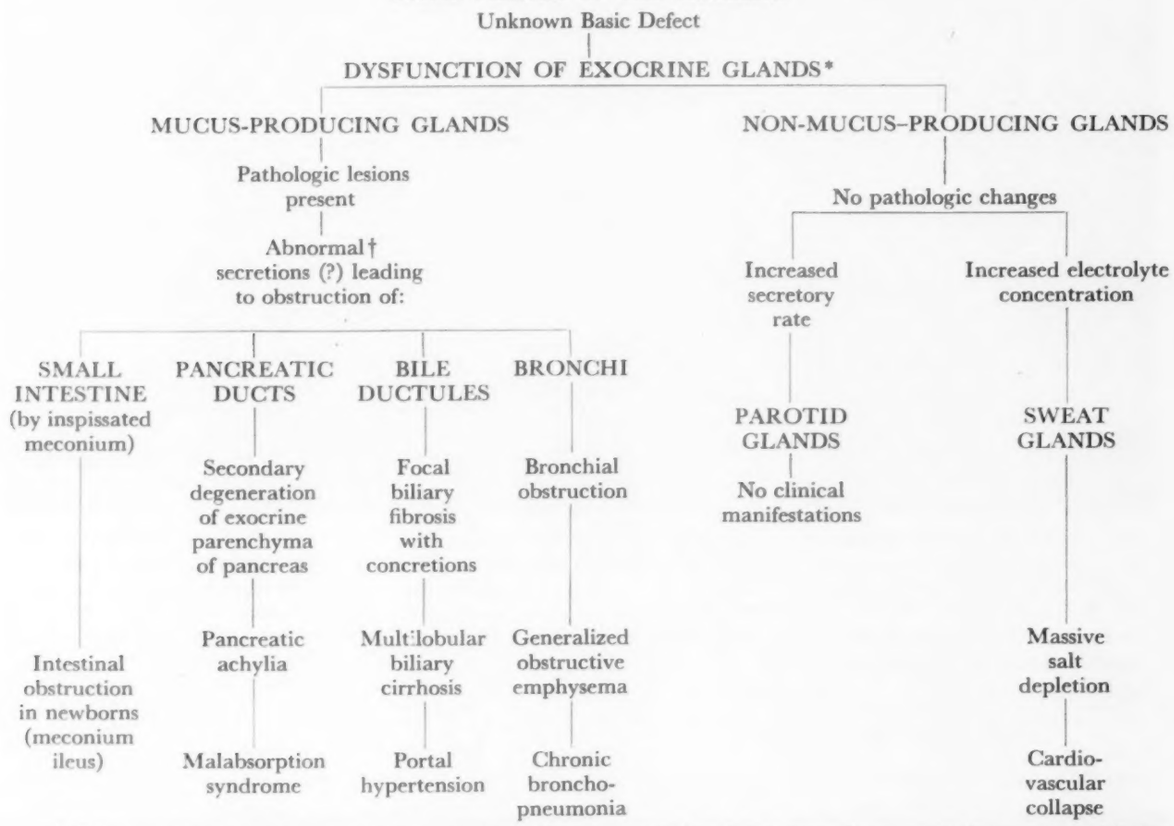
TABLE 1

CYSTIC FIBROSIS OF THE PANCREAS



* Salivary glands also show an increased electrolyte concentration,[50] but have been omitted from the table for clarity as they are mixed (mucus and non-mucus-producing) glands.

† The presence of an abnormal mucoprotein has been shown in duodenal fluid of patients with cystic fibrosis of the pancreas.[48,49]

been reported in Mongolians. Only two of the 397 patients at Babies hospital are Negroes.

Finally it should be stated that fibrocystic disease of the pancreas accounts in the pediatric age group for virtually all cases of pancreatic insufficiency, for the majority of those with chronic pulmonary disease and for a third of children with cirrhosis of the liver and portal hypertension.

Several monographs and review articles have appeared in recent times by Bodian,[5] May,[9] Shwachman and his collaborators[10] and others.[11] While they all cover both clinical and anatomic aspects of the disease, the pathology is especially well illustrated by Bodian.[5] No effort will be made in this article to review all of the papers on cystic fibrosis of the pancreas. Reference will be made only to articles of special interest in connection with aspects of the disorder herein considered.

INVOLVEMENT OF THE DIGESTIVE SYSTEM

PANCREAS. Pathology: On microscopic examination of the pancreas masses of amorphous eosinophilic material are seen obstructing the lumen of acini and ducts (Fig. 1B), many of which are so distended as to have a cystic appearance. Connective tissue is prominent because of replacement fibrosis and condensation of the framework of this organ secondary to atrophy and disappearance of parenchymal cells. Inflammatory changes are prominent in some cases. The islands of Langerhans are normal. These findings are quite similar to those seen in experimental animals after ligation of the ducts.[12]

An important feature of the pancreatic lesion is its progressive nature. The first observable histologic findings in the pancreas are obstruction of an occasional ductule with retention of secretion in the acini which feed into it. (Fig.

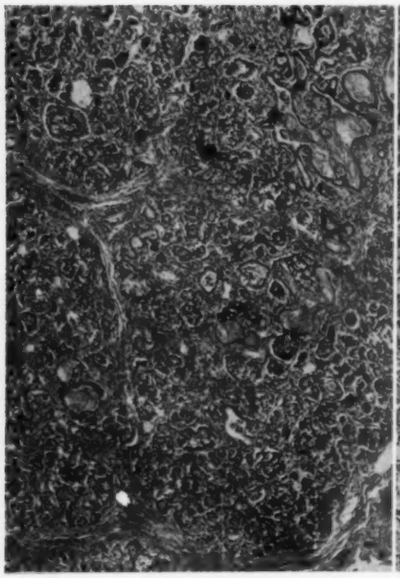


FIG. 1A. Pancreas of patient who died at four months; early stage of lesions. Mild intra- and interlobular fibrosis; ducts and a few acini are plugged by inspissated and at times laminated secretions; mild leukocytic infiltration.

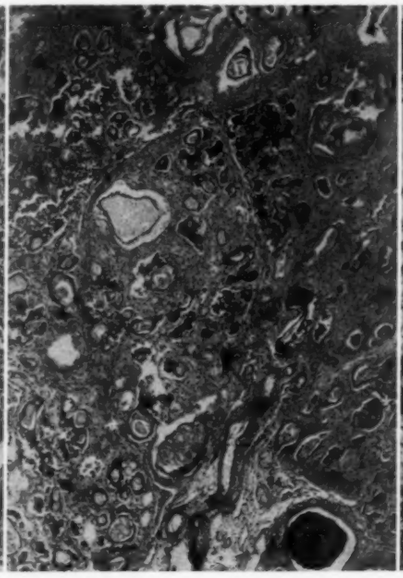


FIG. 1B. Pancreas of patient who died at one year and two months; lesions in advanced stage. Large calcified concretion; almost complete disappearance of acini.

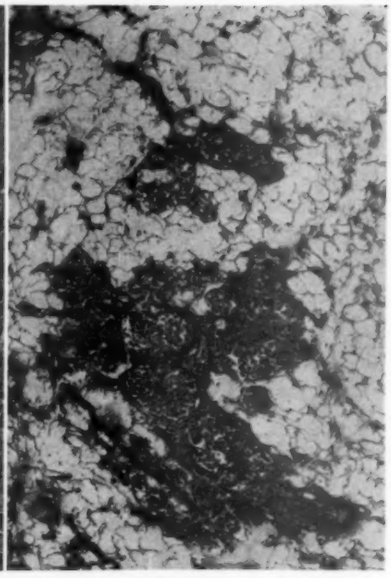


FIG. 1C. Pancreas of patient who died at nine and a half years; late stage. Total atrophy of exocrine parenchyma with persistence of groups of islands of Langerhans; marked lipomatosis and slight fibrosis.

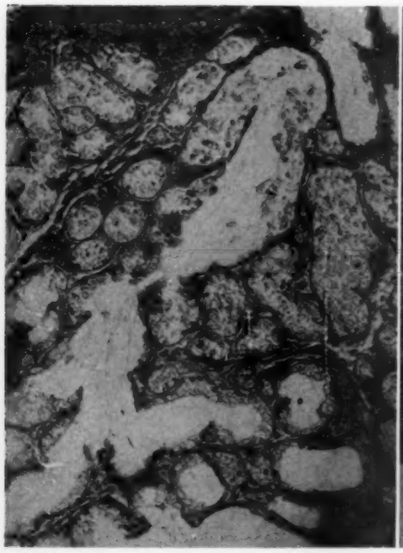


FIG. 1D. Duodenum of patient who died at nine and a half years. Brunner's glands and ducts are distended by an exaggerated amount of secretion.

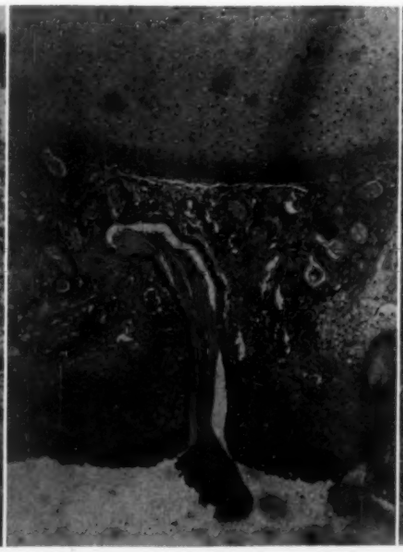


FIG. 1E. Trachea of patient who died at six months. Dense eosinophilic concretion obstructs the duct of a tracheal gland; acute tracheitis.

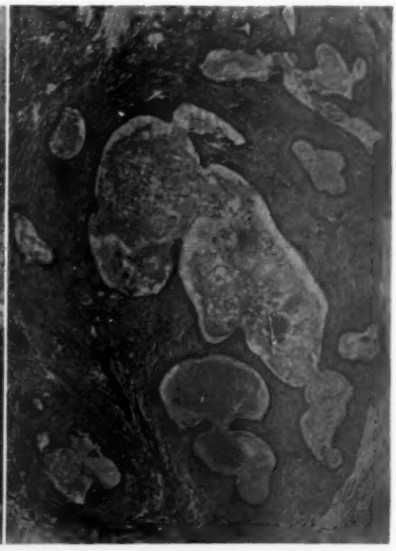


FIG. 1F. Cystic duct of patient who died at two and a half years. All structures are distended by eosinophilic material and the ductal lumen is narrow; there is periductal fibrosis.

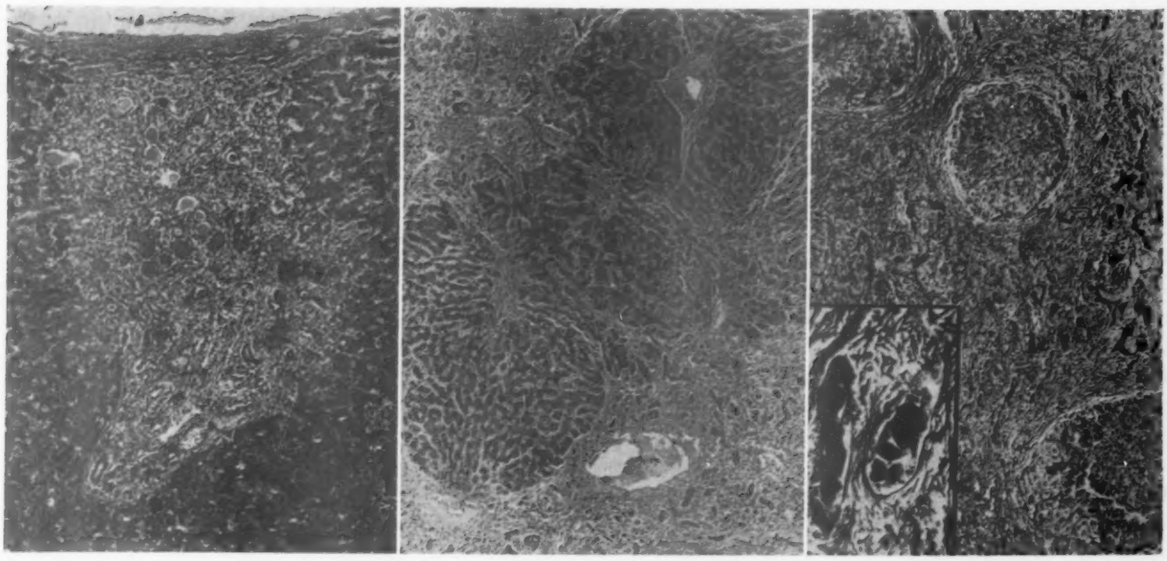


FIG. 2A. Autopsy specimen; focal biliary cirrhosis with concretions in a large subcapsular portal space. Note the thickened, depressed capsule, bile duct proliferation, plugging of the cholangioles with concretions, interstitial inflammation and fibrosis. (From di Sant'Agnese and Blanc.[41])

FIG. 2B. Autopsy specimen; two large foci of biliary cirrhosis merge and a group of four lobules is encircled by fibrous strands extending from the main foci. Superimposed portal changes are present. (From di Sant'Agnese and Blanc.[41])

FIG. 2C. Biopsy specimen of liver; a focus of marked bile duct proliferation with regenerated lobules; many ducts contain eosinophilic concretions (dark spots with hematoxylin and eosin stain). The insert shows a high power view of the concretions (periodic acid-Schiff stain). (From di Sant'Agnese and Blanc.[41])

1A.) These changes extend and increase in severity so that on pathologic examination two or three years after onset of the process little is found except cysts, fibrous tissue and fat (Fig. 1C), with normal looking islands of Langerhans among the rests of pancreatic tissue.

In most patients initial pathologic lesions and pancreatic achylia are present at birth but in some patients involvement of this gland, not present at first, may appear later.[10,13] Even in those infants in whom the lesions in the pancreas are found at birth, the process must have initiated in the latter months of fetal life, as the morphology of the gland is otherwise normal.

Physiopathologic and clinical consequences of pancreatic deficiency: Absence of all three pancreatic enzymes, trypsin, lipase and amylase, leads to impaired absorption of foodstuffs. As a consequence large amounts of fat and nitrogen are present in the stools, as repeatedly shown in metabolic studies.[1,14–17] Digestion of complex carbohydrates is affected to a lesser extent. Although marked differences occur in the degree of steatorrhea and azotorrhea among various patients and in the same patient at various times, as much as 50 per cent or more of ingested fat and protein may be recovered in the stools. The feces are bulky, greasy and extremely foul. Because of the loss of so many ingested calories the appetite is frequently excessive but, despite an adequate intake, malnutrition with retardation in growth and development is an almost constant feature.

While steatorrhea is marked, balance studies by Chung and his collaborators[17] have shown that fat absorption remains proportional to fat intake even at high intake levels. These investigators claim that high dietary fat intake in patients with cystic fibrosis of the pancreas exerts no deleterious effect, an opinion which is in marked contrast with that stated earlier by Andersen[14] and still held by her. Several groups have studied the influence of lack of pancreatic lipase on absorption of fats by means of the rise in serum vitamin A levels after oral administration of a test dose of the vitamin.[18–21] While esterified vitamin A, naturally occurring in fish liver oils, was poorly absorbed, when the compound was administered in the form of the free alcohol, the rise in serum levels of vitamin A was normal. May and Lowe[21] have also shown that finely emulsified vitamin A esters are well absorbed by

patients with fibrocystic disease, a fact which would be in agreement with Frazer's "partition hypothesis" of fat absorption. While only neutral fats would be expected to appear in the stools of such patients, at times a considerable amount of fecal lipid is found in the form of fatty acids. Lipolytic activity of bacteria in the colon has been offered as an explanation.[14] However, an additional factor may be involved. In administering lipiodol orally and measuring urinary excretion of iodine as an indication of splitting of the neutral fat in this compound, Grossman[22] found that in more than half of the patients with cystic fibrosis neutral fats break down to fatty acids despite the absence of pancreatic lipase.

Azotorrhea is marked in patients with cystic fibrosis, as it is in other disorders characterized by involvement of the pancreas.[25,26] Increased stool nitrogen, a constant finding in balance studies, has been shown by the administration of test meals containing I-131-labeled casein[27] to derive from the diet rather than from secretions delivered into the alimentary tract. However, nitrogen balance is positive in most patients with fibrocystic disease,[15,16] presumably because growth is taking place,[28] although at a slow rate, and because of increased dietary protein intake. In contrast to the poor absorption of dietary protein, the absorption of amino acids is unimpaired.[29,30] This difference has been utilized in arriving at a diagnosis.[10,29,30]

Balance studies indicate that the addition of pancreatic extracts has a definite although slight effect in improving fat and nitrogen absorption.[14–16] This contrasts with the marked improvement in the vitamin A absorption test[31] and in the absorption of casein[29] after addition of pancreatin to the test meal.

Complex carbohydrates are not found in the stools in large amounts.[14] No demonstrable impairment occurs in intestinal absorption of simple sugars, and the oral glucose tolerance test is usually normal.[32]

Loss of inorganic ions in the feces is great but is not manifested clinically, presumably because of the compensatory effect of the large amount of food ingested by such patients with its incidental electrolyte content. Also no evidence of deficiency of the water–soluble vitamins is present. However, the great loss of some of the lipo-soluble vitamins with steatorrhea may give rise to serious clinical manifestations. Vitamin A deficiency in the form of xerophthalmia or hyperkeratosis of the epithelium was not uncommon in patients with cystic fibrosis in the past, but is rarely seen at the present time with our improved knowledge of the dietary requirements in this condition. Bleeding and prolonged prothrombin time secondary to vitamin K deficiency are still seen occasionally. Recently Nitowski, Gordon and Tildon[33] have shown by determination of serum levels that vitamin E is frequently deficient in patients with cystic fibrosis, giving rise to marked creatinuria which ceases after administration of this factor. In contrast, rickets due to deficiency of vitamin D is exceedingly rare in patients with cystic fibrosis. Lack of growth has been repeatedly cited as the reason for this immunity but it actually provides an unsatisfactory explanation since growth in this disease is usually only retarded and at times may be almost normal, even in the face of marked steatorrhea.

Rare occurrence of diabetes mellitus: Just as in experimental dogs in which the pancreatic ducts are ligated,[12] in patients with cystic fibrosis the endocrine function of the pancreas is generally not affected. Diabetes mellitus, while rare, does occasionally occur. In this clinic we have encountered only one such case in 397 patients. Others have had similar experience.[10] As in adults having undergone total pancreatectomy[26] or with chronic recurrent pancreatitis, the diabetes is mild. In the one patient still living who was observed by the author, the pancreas was unmistakably visualized by fine calcifications upon roentgenographic examination of the abdomen. Calcifications are found in this organ on pathologic examination (Fig. 1B) but in the author's experience have never been of sufficient size to be visible roentgenographically, with this one exception. With the amount of fibrous tissue present and its inevitable crowding of the islands of Langerhans, it is surprising that diabetes mellitus is not encountered more frequently.

Normal or partial pancreatic function: All the clinical and pathologic observations summarized thus far apply to patients with cystic fibrosis and pancreatic achylia. In the past, pancreatic deficiency was considered the basic defect in this disorder and the diagnosis of fibrocystic disease was withheld unless absence of pancreatic enzymes could be demonstrated. With the advent of the "sweat test" the situation has changed and it is now possible to confirm what had previously only been suspected.[34,35] Clinical, metabolic and pathologic data were presented by this author in 1955[13] showing that there

are some patients with this disease in whom no pancreatic involvement occurs but who present other features of the disorder, particularly the characteristic abnormality in the sweat electrolyte pattern. The same studies showed that in patients in whom pancreatic function is normal pancreatic achylia may later develop and that in some instances pancreatic function is still present but depressed. It is estimated[13] that 10 per cent of patients with fibrocystic disease have either normal or partially preserved pancreatic function.

Patients with cystic fibrosis who have normal pancreatic function, as might be expected, do not have any of the clinical manifestations consequent upon absence of pancreatic enzymes, namely, malnutrition, retardation in growth, excessive appetite and abnormal stools. If the pancreas is still functioning but to a reduced degree, these symptoms are present to a variable degree.

The implications as to the laboratory tests dependent upon pancreatic function are also clear. The presence of normal amounts of pancreatic enzymes on duodenal assay, the absence of steatorrhea or azotorrhea, or a normal vitamin A absorption curve do not *per se* negate the diagnosis.

LIVER. The occasional finding at autopsy of localized foci of biliary obstruction and fibrosis was recognized from the first descriptions of cystic fibrosis of the pancreas.[2,36–40] Only recently has it been realized, however, that such initial lesions of the liver are common[5] and at times lead to severe clinical manifestations which may dominate the clinical picture.[41]

Pathology: Changes have been found in the liver at necropsy as early as three days of life but appear to become more common and more extensive with advancing age. On inspection of the capsule the initial lesion is seen as a varying degree of pitting, corresponding to small, stellate, depressed cirrhotic foci. Microscopically (Fig. 2A), bile ductules are plugged with concretions of amorphous eosinophilic material and are surrounded by an area of fibrosis, biliary proliferation and inflammatory reaction. Bile staining is not marked. The nature of the concretions is not clear but they show a morphologic and histochemical resemblance to those in the pancreas. Both may be interpreted as resulting from inspissated secretions. Their presence is characteristic and diagnostic of fibrocystic disease of the pancreas. The designation focal biliary cirrhosis with concretions has been given to this first stage of the process. Similar lesions have been found in 25 per cent of cases in two autopsy series and in virtually all of the patients more recently examined.[5,41]

With time multiple foci emerge. Diffuse portal changes take place. Multiple lobules are encircled and trapped by the fibrotic process. (Fig. 2B.) As fibrosis becomes more extensive, the architecture of the liver is destroyed and the original foci of concretions may on occasion even disappear. Because of the focal character of the initial lesions, damage proceeds irregularly, and areas of preserved lobular pattern are found adjacent to massive foci of bile duct proliferation with fibrosis. Always characteristic is the limited bile stasis. The term multilobular biliary cirrhosis with concretions (Fig. 2C) has been used to indicate the distinctive character of this process.[41]

Only at this late stage is the spleen enlarged. It shows the chronic passive congestion with pulp cord fibrosis seen in portal hypertension.

Clinical manifestations: The lesions of focal biliary cirrhosis give rise to no clinical manifestations even when extensive. Only in patients in whom progression to the diffuse type of multilobular biliary cirrhosis occurs does the liver become hard and nodular. After a varying length of time, a few months or a few years, distortion and remodeling of the parenchyma of the liver is such that portal hypertension eventually develops.[41] It is only at this stage that the condition becomes clinically manifest with the appearance of hepatosplenomegaly, hypersplenism, gastrointestinal bleeding or ascites, or a combination of the three. Liver function tests may give abnormal results at this time, but characteristic of this process is the absence or only slight degree of icterus.

Etiology and incidence: The histologic appearance suggests that the cycle is initiated by primary mechanical obstruction of bile ductules by what appear to be inspissated secretions, an expression presumably of the abnormality of many secretory products in this generalized disease. While focal biliary cirrhosis is a common postmortem observation in patients with fibrocystic disease, diffuse multilobular biliary cirrhosis with clinical manifestations is rare. The author has seen only seven such patients in the present series of 397 cases, accounting, however, for one-third of children with portal hypertension.[41] What, then, triggers the passage from one type of hepatic lesion to the other?

As shown in recent studies[41] it is difficult to

incriminate any single factor in the elicitation of this response. It may reasonably be postulated that added infection (for example, infectious hepatitis) or nutritional insult or possibly other noxious agents (continued antibiotic therapy, ascending cholangitis, and the like) might cause an adverse response on the part of the liver which is already basically abnormal.

Fatty degeneration of the liver, not uncommonly seen in the past in patients with cystic fibrosis, is now seldom encountered, presumably because of improved knowledge of the dietary requirements of such children. It should be kept in mind that in fibrocystic disease only the exocrine portion of the pancreas is non-functional, but judging from the nutritional status of patients and from autopsy material there is no reason to believe that lipocaic, choline, methionine or other specific therapy is needed to prevent fatty deposits in the liver. These agents are required for the welfare of experimental pancreatectomized dogs and sometimes for patients who have been subjected to total pancreatectomy.[26]

It is recognized in other situations[42] that a relation exists between the non-functioning pancreas and abnormalities in the liver. However, it is important to realize that in persons with cystic fibrosis the liver may at times be affected even if pancreatic function is normal.[41] A definite relationship between involvement of the two organs is not clear, therefore, and it can be argued that hepatic and pancreatic changes are independent of each other and represent an expression of the unknown generalized basic defect.

MECONIUM ILEUS. At birth in from 10 to 15 per cent of all patients with cystic fibrosis of the pancreas signs of obstruction of the small intestine are shown by thick, tenacious meconium, the so-called meconium ileus. The association of this picture with pancreatic achylia was first pointed out by Andersen[2] in 1938 and later by Farber.[43]

In meconium ileus the lower ileum is obstructed by rubbery, greyish meconium. Proximal to the obstruction is a large amount of viscid, abnormal looking material greatly distending the small bowel and causing it at times to twist upon itself so as to simulate a volvulus. Distal to the obstructed segment the colon is small. On occasion the obstructed bowel perforates in utero, giving rise to meconium peritonitis. It should be realized, however, that any obstruction in the intestinal tract (for example, atresia), not meconium ileus alone, may cause passage of meconium into the peritoneal cavity.

Successful surgical treatment of this condition was first reported by Hiatt and Wilson in 1949.[44] In recent times Gross[45] has reviewed the surgical aspects. In patients who survive surgery other manifestations of cystic fibrosis eventually develop. While the pathogenesis of meconium ileus has been related to pancreatic achylia occurring before birth,[43] there is no evidence that in these patients an unusually severe type of fibrocystic disease is later manifested. The subject has recently been well reviewed by Shwachman.[46]

CHEMICAL STUDIES OF INTESTINAL SECRETIONS. An abnormality of mucous secretion has been postulated repeatedly[5,10,36,47] in order to explain many of the pathologic findings and clinical symptoms of cystic fibrosis of the pancreas. However, little evidence in support of this hypothesis has been presented. In recent studies performed at the Babies Hospital[48,49] a physico-chemical difference has been demonstrated in the behavior of mucoproteins in the duodenal content of patients with cystic fibrosis of the pancreas as compared with that of control subjects. In the latter, mucoproteins following precipitation with a mixture of ethanol and benzene are readily soluble in water. In patients with cystic fibrosis of the pancreas a considerable part of the precipitated glycoprotein cannot be redissolved in water or brought into solution by the action of trypsin. On analysis the carbohydrate moiety of this water-insoluble mucoprotein present in patients with fibrocystic disease is found to be different in certain chemical characteristics from the water-soluble fraction.

As seen in Table II the water-insoluble mucoprotein was found in forty-seven of fifty patients with fibrocystic disease irrespective of whether or not pancreatic function was preserved. Abnormal mucoprotein was absent in seventy-six of eighty-one control patients, both children and adults. In the latter group were included normal persons and a variety of subjects with conditions other than cystic fibrosis. Of the five "control" patients in whom the water-insoluble mucoprotein was found, two were suspected on clinical grounds of having fibrocystic disease. Two others in this same group presented an unclassifiable nutritional disorder, with abnormal stools, steatorrhea and malnutrition, but no pulmonary involvement was noted.

In this early stage of the investigation little can be said in this connection. It is possible that a similar abnormality may be present in mucous secretions elsewhere in the body, and studies in this direction are being made. The absence of abnormal mucoprotein in the duodenal contents of three of fifty patients with cystic fibrosis was not thought surprising in view of the variable involvement of various organs in this disease. By the same token, as either the sweat glands or the pancreas have been found not to be affected in some instances, it is quite possible that some of the "controls" did have fibrocystic disease but without involvement of both of these areas.

TABLE II

OCCURRENCE OF WATER-INSOLUBLE MUCOPROTEIN IN DUODENAL CONTENTS OF PATIENTS WITH CYSTIC FIBROSIS OF PANCREAS AND CONTROLS

Group	No. of Patients	Age Range (yr.)	Involvement			Water-insoluble Mucoprotein
			Pancreas	Lungs	Sweat Glands	
Cystic Fibrosis of Pancreas						
I	39	½–12	+	+	+*	+
II	7	½–7	0	+	+	+
III	1	2	+	+	0†	+
IV	3	½–5	0	+	+	0
Total	40					
Control Patients						
A	61	½–13	0	0	0‡	0
B	12	25–73	0	0	not performed	0
C	3	39–66	+§	0	not performed	0
D	5	½–49	0	0	0	+
Total	81					

* Sweat test performed in only nineteen of thirty-nine cases.
† Only patient with cystic fibrosis and normal sweat electrolytes of 140 patients tested.
‡ Sweat test performed in only thirty-eight of sixty-one cases.
§ Diagnosis in the three cases: carcinoma of pancreas in one, chronic pancreatitis in two.

The duodenal contents in patients with cystic fibrosis frequently are found to be abnormally viscous;[10,35] indeed, increased viscosity may be the first manifestation of the disease, at times preceding pancreatic enzyme deficiency. Increased viscosity is therefore an important finding, although its significance remains obscure, since it seems to vary independently of pancreatic function.

Sodium, chloride and potassium concentrations in the duodenal contents of patients who have and in those who do not have pancreatic deficiency and in control persons with a variety of other conditions appear to be identical.[50]

Finally, some studies should be mentioned bearing on the composition of the meconium in meconium ileus. In 1952 Rapoport and Buchanan[51,52] reported that material from such a patient contained much more nitrogen and less carbohydrate than normal meconium with which it was compared. This was thought to be accounted for by lack of proper digestion because of the absence of pancreatic trypsin. The increased viscosity of the abnormal meconium was attributed to its higher mucoprotein content. Glanzmann in 1950[53] also studied meconium from a patient with meconium ileus. He found that the diseased meconium was able to form a gel-like mass with lipids in the presence of water, and he thought that this might be due to the presence of an abnormal protein.

INVOLVEMENT OF RESPIRATORY TRACT

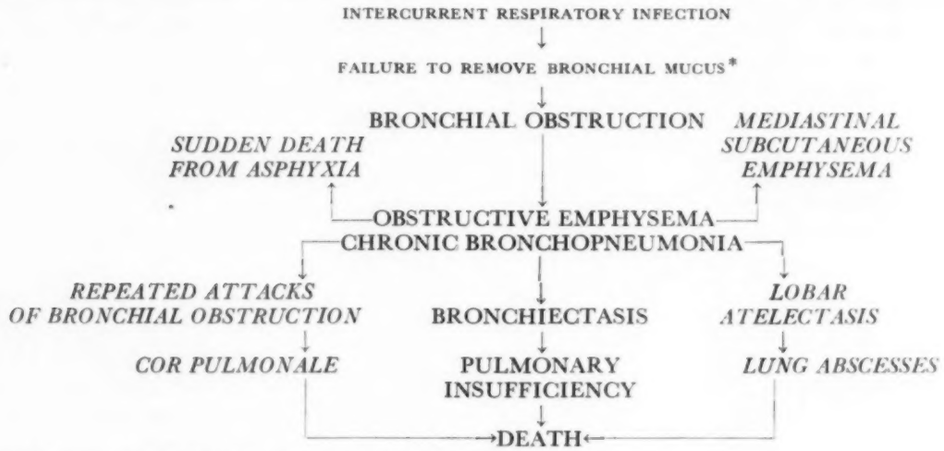
At some time in their illness virtually all patients have respiratory involvement,[54,55] usually with onset in infancy or early childhood, but symptoms may appear at different times. Varying also are the severity of the lung involvement and the duration of single phases of respiratory infection. Frequently the pulmonary disease is severe; in this clinic it has accounted for over 90 per cent of the 173 deaths.

For weeks or months the patient has a dry, hacking, non-productive cough. Then (Table III), usually following an intercurrent respiratory infection, there is failure to remove properly the excessive bronchial mucus produced, perhaps because of its abnormal structure or physicochemical behavior. Generalized bronchial obstruction follows and at times is severe. After a variable but usually short period, the infection, until then mild and perhaps localized in the main divisions of the bronchi, becomes widespread and frequently fulminating by invasion of the obstructed air passages down to their smaller subdivisions. Respiratory distress is present and there may be marked anoxia, carbon dioxide retention and air hunger. The patient is frequently quite ill, presenting the picture of severe generalized pulmonary infection.

Thanks to effective antibiotic agents, spread of the infection to the blood stream and occurrence of massive lobar atelectasis,[56] such as has been seen in the past, is now usually not present.

TABLE III

COURSE OF SEVERE RESPIRATORY DISEASE AND ITS COMPLICATIONS IN CYSTIC FIBROSIS OF PANCREAS



* Failure to remove bronchial mucus is probable but not proved.

Secondary bronchopneumonia is brought under temporary control but some degree of bronchial obstruction persists. The cycle is then repeated with subsequent respiratory infections, giving rise to the characteristic course of the disease punctuated as it is by repeated episodes of increased bronchial obstruction and secondary infection. Any one of these relapses may be fatal.

On physical examination the thorax is barrel-shaped and the percussion note tympanitic. On auscultation rales may be heard all over the pulmonary area, disappearing after a coughing spell. Clubbing of fingers and toes is usually marked. On roentgenograms the signs of generalized obstructive emphysema are visible, namely, increase of the anteroposterior diameter of the chest, small cardiothoracic index and depression of the leaves of the diaphragm. The signs of bilateral bronchopneumonia are present. If the pulmonary infection is of moderate severity, the lungs present a "honeycomb" appearance in x-ray films; in the more advanced state they may show a "snow flake" picture similar to that of generalized pulmonary tuberculosis. Peribronchial "cuffing" is very much in evidence, even early in the illness.

Staphylococcus aureus hemolyticus was consistently recovered in the past from nose and throat cultures and at autopsy from the lungs either in pure culture or as the predominant organism.[57] The bacterial flora of the nasopharynx and of the bronchi has been altered in recent years by the use of wide spectrum antibiotics, and proteus, Pseudomonas aeruginosa, other bacteria and Candida albicans may be found on occasion. Still the basic and recurrent infection is due to staphylococcus, a striking association of this organism with cystic fibrosis. This finding is the more notable as such patients resist Staphylococcus aureus infection elsewhere in the body (for example, the skin) quite normally.

The pulmonary component of cystic fibrosis is an intrabronchial disease, and bronchial obstruction is its primary and cardinal manifestation.[11,55,56] Secondary infection may never cause permanent damage to the bronchial wall, leading only to mild chronic disease of the lung which is kept in check effectively by broad-spectrum antibiotics.[58] If irreversible damage is sustained by the bronchi, bronchopneumonia is progressive and eventually leads to the distressing picture of pulmonary insufficiency and death through various complications. (Table III.) The duration between onset of disease and its fatal termination varies from a few weeks to as long, in the author's experience, as fourteen years; the average is two or three years.

It is important to keep in mind that involvement of the lungs in cystic fibrosis of the pancreas may be initiated by measles or pertussis. Both of these conditions frequently give rise to bronchial complications even in otherwise normal persons.

INVOLVEMENT OF SWEAT AND SALIVARY GLANDS

Sweat Glands. In 1953 in a series of studies in this hospital[8,59,60] it was shown that the sweat

of patients with cystic fibrosis of the pancreas consistently has a concentration of chloride and sodium two to five times that of normal persons and of patients (children and adults) with a variety of other conditions. Sweat potassium also was affected but to a lesser degree. Chronic disease of the lung, diabetes mellitus, acquired pancreatic deficiency, cirrhosis of the liver and many other disorders do not *per se* change the electrolyte concentration of sweat. Sweat electrolyte values in these and subsequent investigations (Fig. 3) were as follows: (1) patients with cystic fibrosis, chloride 50 to 160 mEq. per L. (mean 106 mEq./L.), sodium 80 to 190 mEq. per L. (mean 133 mEq./L.); (2) control subjects, chloride 4 to 60 mEq. per L. (mean 27 mEq./L.), sodium 10 to 80 mEq. per L. (mean 52 mEq./L.).

Metabolic studies[60] indicated that the abnormally high sweat electrolyte level in patients with fibrocystic disease was not significantly affected by administration of desoxycorticosterone acetate, dietary salt restriction or exposure to hot weather. Function of the kidneys and adrenal glands in such patients was found to be normal. It was concluded, therefore, that the defect was in the sweat glands themselves. No explanation has been offered to date as to physiopathology responsible for this phenomenon. In the original studies sweat was collected in limited areas of the trunk[8,59,60] and presumably was only from eccrine glands. Recently, however, Cooke[61] has found a similar difference in total body sweat between patients with cystic fibrosis and control subjects.

These findings were subsequently amply confirmed by others.[10,62,63] So far we have seen only one patient (of 140 tested) with cystic fibrosis of the pancreas and normal sweat electrolytes. A similar ratio has been found in other clinics.[64]

While this specific abnormality of sweat electrolytes has not been found as yet in any condition other than fibrocystic disease, about 20 per cent of relatives of patients known to have the disease (parents and siblings) have an increased concentration of chloride and sodium in the sweat.[8,13] Usually these patients have no clinical manifestations of the disease but at times chronic disease of the lung is present, although pancreatic function is normal.

These findings indicate that cystic fibrosis is in reality a generalized disease in which many and perhaps all exocrine glands, mucus producing and others, are affected. The occurrence of sweat electrolyte abnormalities in relatives of patients known to have the disease demonstrates the existence of mild and incomplete forms. Knowledge of the markedly increased concentration of salt in sweat and of the inability

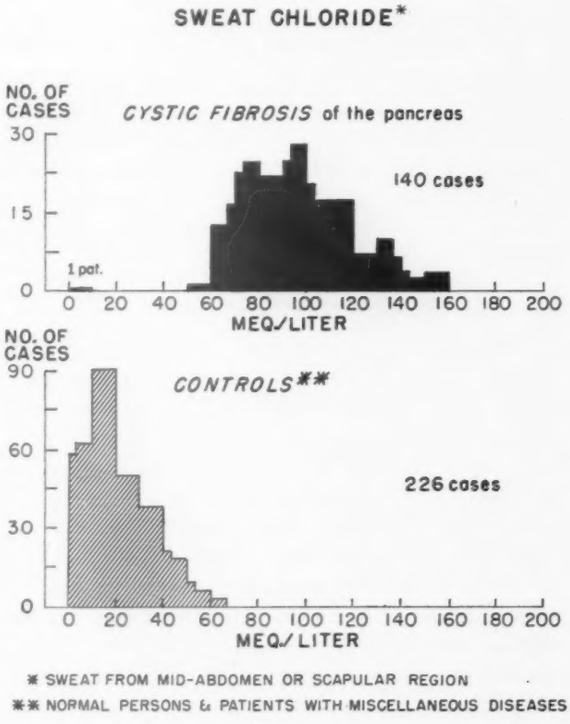


FIG. 3.

of these abnormally high levels to be decreased significantly in patients with fibrocystic disease explains the unusual susceptibility of such patients to hot weather.[65] Massive salt loss through sweat leads to salt depletion, cardiovascular collapse and, at times, death.[60] From a practical standpoint this knowledge has pointed the way to prevention and treatment of these complications. In addition, the specificity of the "sweat test" has led to its widespread use in diagnosing cystic fibrosis of the pancreas.

Salivary Glands. In cases of fibrocystic disease, compared with normal, the concentration of chloride and sodium is also abnormally increased in mixed saliva.[11,50,60] Potassium is not significantly different. In the figures obtained in this laboratory the overlap in values is considerable in the two groups; it was therefore considered by the author that salivary electrolyte determinations, while of theoretic interest, could not be used as a diagnostic tool. Prader and

Gautier[66] in a small number of patients found a clear-cut difference between the chloride and sodium levels in the saliva of patients with cystic fibrosis and others. Their values, however, represented the average of six determinations. In view of the variability in composition of saliva following various stimuli it is clear that much further work is needed.

At the same time it was also shown by us[11,50,60] that the parotid secretory rate is greatly increased in patients with cystic fibrosis as compared with that of control subjects. This finding is difficult of interpretation but the parotid secretory rate has been used[67] to evaluate the degree of activity of the autonomic nervous system.

GENETIC STUDIES

The first comprehensive study of the genetic aspects of cystic fibrosis of the pancreas was reported by Fanconi and Botztein[68] in 1944, who recorded the family trees of twenty-five children with this disease. No definite conclusion was reached. Andersen and Hodges[6] in 1946 published data on forty-seven siblings and family trees for twenty of them. They concluded that hereditary transmission by a recessive mechanism was likely. Because of the occurrence of the disease in the majority of siblings in a fair proportion of the affected families and because a lethal simple recessive trait would tend to disappear rapidly, when not too frequent, Andersen and Hodges concluded that an uncomplicated recessive trait dependent on a single gene was unlikely. In 1949 Lowe, May and Reed[7] presented data on 118 patients, nine of them with the family trees. They felt strongly that the evidence at that time indicated that cystic fibrosis resulted from a single recessive gene which in the homozygous condition caused the fully manifested clinical picture. In 1952 Carter,[69] from data on 116 children observed in London, also expressed the view that the recessive gene hypothesis for the transmission of this disease was satisfactory, although not completely so.

The situation was altered with the demonstration in 1953 in this clinic[8] that in a significant proportion of relatives (siblings and parents) of patients known to have the disease the elevation of sweat electrolytes was characteristic of that of children with cystic fibrosis of the pancreas, without other manifestations of the disorder. The author's data show[8,32] that a majority of such persons are grouped within relatively few families. It may also be significant that many of these show sweat electrolyte values which are somewhat lower than those found in patients with the full-blown picture of the disease.

This certainly suggests that we are dealing with more than one factor. Perhaps the most acceptable hypothesis at this time, according to Childs,[70] is that presupposing a gene or genes which cause overt disease in homozygotes and give partial expression or no expression of the disorder in heterozygotes.

CLINICAL PICTURE AND DIAGNOSIS

In the majority of patients the combination of symptoms of intestinal insufficiency and chronic disease of the lung suggests the diagnosis. However, as already indicated this protean disease is characterized by variable involvement of the areas affected and patients are seen at times in whom the classical and easily recognizable clinical picture is not presented. The following groups can be differentiated (Table IV):

Group I consists of patients presenting a combination of intestinal and respiratory manifestations. This category accounts for the majority of cases.

Group II consists of patients presenting pancreatic insufficiency but no lung involvement. Such cases are not uncommon and frequently are mistakenly thought to represent idiopathic celiac disease or other conditions leading to steatorrhea in infants and children. Even in these children the large, foul stools, the excessive appetite and the malnutrition that is present despite apparently adequate dietary intake should lead one to suspect the correct diagnosis.

Group III consists of patients with chronic disease of the lung in whom intestinal symptoms are lacking. In about 10 per cent of cases the function of the pancreas is either normal or slightly impaired and symptoms referable to deficiency of this organ are therefore lacking. These patients present only chronic disease of the lung, which must be differentiated from the other conditions that result in similar clinical manifestations in this age group. Of assistance in diagnosis is the fact that the majority of children with generalized obstructive emphysema, especially if they are Caucasian, have cystic fibrosis.

Group IV consists of children in whom cirrhosis of the liver with portal hypertension dominates the clinical picture. At the Babies Hospital seven children have been seen in whom

TABLE IV

CLINICAL TYPES OF CYSTIC FIBROSIS OF PANCREAS

Name	Sex and Race	Age (yr.)	Involvement					Duodenal Assay Tryptic Activity* (u./cc.)	Fecal		Sweat† (mEq./L.)	
			Pancreas	Lungs	Sweat Glands	Liver	Diabetes Mellitus		Nitrogen (% intake absorbed)	Fat (% intake absorbed)	Cl	Na
Usual Type of Cystic Fibrosis of Pancreas												
		children	+	+	+	0	0	absent	decreased	decreased	increased	
Clinical Variants of Cystic Fibrosis of Pancreas												
J. P.	F, W	3	0	+	+	0	0	569	97	98	97	120
M. K.	F, W	3	±	+	+	0	0	85	81	90	110	121
A. R.	F, W	11	+	+	+	+§	0	0	..	..	72	84
P. D.	M, W	14	+	0	+	0	0	0	..	60	89	89
J. H.‡	M, W	1½	+	+	0	0	0	0	..	..	8	7
M. H.	F, W	6	+	±	+	0	+	0	..	..	103	105

* Normal = 100 viscosimetric units/ml.; average = 250 u./ml.

† Normal values (mEq./L.) = sweat Cl 4 to 60 (mean 27), sweat Na = 10 to 80 (mean 52).

‡ Only patient with cystic fibrosis of the pancreas, but normal sweat electrolytes of 140 patients tested.

§ Cirrhosis of liver with portal hypertension.

cirrhosis of the liver was clinically manifested. In two of these, cystic fibrosis was not suspected prior to admission, and the symptoms of portal hypertension dominated the clinical picture. Hospitalization in both instances was for gastrointestinal bleeding.[41] Neither one had ever had respiratory symptoms previously, although these manifestations developed subsequently in both.

Group v consists of heat casualties in whom clinical manifestations of the disease were not noted previously. In two patients, acute salt depletion during hot weather necessitated admission to the hospital for emergency treatment. Cystic fibrosis, previously not suspected, was subsequently diagnosed in both children. In one instance pancreatic achylia was present which had given rise to only minimal symptoms; in the other the pancreas was not involved.

All of these patients have in common the increase in sweat chloride and sodium characteristic of cystic fibrosis, so that determination of sweat electrolytes is of assistance in arriving at a diagnosis. The "sweat test" is a valuable diagnostic tool and one which is not dependent on pancreatic function. The introduction of plastic bags to make the patients sweat[10] has greatly simplified the procedure, and the analysis involved is within reach of most hospital laboratories.[59,60] For diagnostic purposes the determination of sweat potassium is generally not performed because the overlap in values between patients with cystic fibrosis and those with other conditions impairs its diagnostic usefulness.

In the past, definitive diagnosis has rested on the demonstration of absence of pancreatic enzymes on duodenal assay. However, it is now known that their presence does not negate the diagnosis of fibrocystic disease, and this test is of little or no help in patients in whom pancreatic function is normal or partially preserved. The same can be said of many of the other tests devised to diagnose this disease[10,29,30,71,72] which are dependent for their results on pancreatic achylia.

The demonstration of the presence in the duodenal content of a water-insoluble mucoprotein[48,49] may prove to be of diagnostic help in certain patients. It is too early in the investigation to state this with assurance.

The diagnosis of cystic fibrosis of the pancreas should then be based on the following criteria, with the understanding that some of them may be absent in the single patient.

1. Pancreatic insufficiency, present in 90 per cent of patients, leads to steatorrhea, azotorrhea and malnutrition. On duodenal assay pancreatic enzymes are absent. In our laboratory we have relied mainly on the determination of tryptic activity of duodenal fluid.[73]

2. Pulmonary involvement is characterized by generalized obstructive emphysema and chronic bilateral bronchopneumonia. Respiratory involvement of varying degrees of severity is found in almost all patients at some time during the disease.

3. Abnormal sweat, showing on "sweat test" the characteristic increase in sweat chloride and sodium concentrations, leads at times to salt depletion and even to death in hot weather. Of 140 patients with fibrocystic disease who have been tested to date, only one was found to have normal sweat electrolytes.

4. A family history which reveals the presence of siblings with the same condition is of diagnostic assistance. Their absence does not rule out the disease.

5. Cirrhosis of the liver gives rise to hepatosplenomegaly and the symptoms of portal hypertension, namely, hypersplenism, gastrointestinal bleeding or ascites, or a combination of the three. A biopsy specimen of the liver reveals multilobular biliary cirrhosis, and at times "concretions" are found. (Fig. 2C.)

It is evident, therefore, that cystic fibrosis of the pancreas must be differentiated in many instances from other conditions giving rise to steatorrhea, chronic disease of the lung or cirrhosis of the liver with portal hypertension in the pediatric age group.

PROGNOSIS

The pathologic changes in the pancreas and the effects of pancreatic deficiency attracted attention first and gave the disease its name. However, involvement of the respiratory tract usually dominates the clinical picture and determines the fate of the patient.[10,11,54,55]

The basic difficulty in pulmonary involvement in cystic fibrosis of the pancreas is in removal of bronchial mucus, which obstructs the air passages and leads to secondary bronchopneumonia. The walls of the bronchi are not affected at first but are involved if the secondary infection is sufficiently long or severe. If irreversible damage to the bronchial wall occurs the disease of the lung becomes slowly progressive and eventually leads to death (Table III) in a period varying from a few months to more than ten years but averaging two to three years. Antibiotics cause only temporary remission in chronic lung involvement, and fail to have lasting effect. If permanent damage to the bronchi does not occur the pulmonary involvement remains mild and is kept in check by antibiotics. After the age of ten most such patients improve,[11,55] perhaps because of the increase in size of the pulmonary structures and the consequent mechanical favorable effect on bronchial obstruction. In such patients the degree of clearing by roentgenograms is surprising, and pulmonary function tests can be virtually normal.[58]

Although less important to the patient's ultimate outlook, attention should be paid to the effects of pancreatic deficiency. Maintenance of good nutrition, improvement in the character of the stools and avoidance of vitamin deficiencies are essential to the patient's welfare. In recent years, with improved knowledge of these patients' nutritional requirements, the consequences of pancreatic achylia have not governed the patient's outlook.

It is believed by some authors[74,75] that maintenance of a dietary regimen specifically aimed at overcoming the effects of pancreatic insufficiency has a favorable effect on the incidence and course of pulmonary involvement. In the writer's opinion and in the opinion of others[9,10] the evidence is not too convincing. In many patients who were given a strict diet before onset of pulmonary manifestations chronic disease of the lung, at times severe, progressive and fatal has subsequently developed. Furthermore, now conclusive evidence has appeared[8,13] that the lungs may be affected in patients in whom pancreatic function is normal and in whom digestive symptoms are entirely absent. It must be concluded that involvement of the pancreatic and pulmonary areas is independent and one has no effect on the other, except insofar as the patient in a satisfactory state of nutrition is better able to cope with infection.

Meconium ileus and the consequent intestinal obstruction presents a hazard to 10 per cent of patients in the newborn period. About half survive operation and then face the same uncertain outlook as other children with cystic fibrosis. Massive and at times uncontrollable gastrointestinal bleeding consequent upon por-

tal hypertension due to cirrhosis of the liver presents an additional danger in some instances. Finally, massive salt depletion through sweat in hot weather has led at times to severe and even fatal consequences.

Of 397 patients, 173 are dead. More than 90 per cent of the deaths have been due to chronic disease of the lung. Thirty-five patients are above the age of ten, the oldest being twenty-one years. As a whole these older patients are doing well,[76] two of them have entered college and three others are working at full time jobs. It is probable that at least some of them will suffer from chronic pulmonary difficulties in adult life.

TREATMENT

Treatment of patients with cystic fibrosis of the pancreas is directed towards correcting the effects of pancreatic deficiency by dietary measures and controlling the respiratory infection by administration of antibiotics.

The principles of diet therapy as recommended by Andersen[75] are as follows: The diet is of high caloric, high protein, low fat and moderate starch content. Simple sugars and liposoluble vitamins are given in liberal amounts. Pancreatic extracts are administered with each meal. Patients with cystic fibrosis absorb protein as inefficiently as fats but such children tolerate increased amounts of dietary protein, thereby achieving a positive nitrogen balance. Further details as to this type of diet are given in the same paper.[75] In view of the abnormal sodium chloride content of sweat in these children and its possible adverse effects on the patients in hot weather,[8,59,60] liberal use of the salt shaker is advised at all times; two extra grams of salt are given in summer.[11]

The question as to whether patients with cystic fibrosis and pancreatic achylia need a restricted diet or not has led to a great deal of controversy. On the one hand some investigators, notably Andersen,[74,75] feel it very important to keep all patients with pancreatic deficiency on a strict dietary regimen. On the other, some authors[9,17,54] have expressed the view that patients with fibrocystic disease need to compensate for the loss of calories through steatorrhea and azotorrhea, but that within these limits their intake need not be limited. Some of the metabolic data[17] would tend to support the former hypothesis, others the latter.[14] The author would like to take an intermediate position, recognizing that further metabolic studies should be made before the question is finally settled. The effects of lack of pancreatic enzymes vary considerably even among patients with pancreatic achylia. Some need virtually no dietary restriction, as shown by their growth, state of nutrition and the character of the stools. In others, a dietary regimen is imperative. As there is no way of determining beforehand to which category any particular patient will belong, it is suggested that a strict dietary regimen be instituted and then gradually liberalized as the clinical manifestations permit. It goes without saying that patients with cystic fibrosis, but without pancreatic involvement, need no dietary restrictions.

The necessity for antibiotic treatment of patients with pulmonary involvement is universally accepted, and there is general agreement as to the agents to be used. In 1944 in this clinic penicillin by the intramuscular and the inhalational routes was introduced.[57] As other more effective antibiotics became available they came into general use[10,11,77,78] with beneficial results.

Antibiotics are given in an intensive course lasting from seven to fifteen days and frequently require hospitalization. If evidence of lung involvement persists (as is usually the case) broad-spectrum antibiotics are given in prophylactic dosage for months or years. If, on the other hand, evidence of pulmonary disease no longer persists these agents can be discontinued. The prolonged administration of oral antibiotics is undesirable, and the danger of emergence of bacterial resistance is recognized; however, administration of these drugs over a long period is frequently imperative. Further details as to dosage and route of administration are given elsewhere.[11]

Surgery is required to relieve intestinal obstruction due to meconium ileus[45,46] and surgical shunting procedures may be needed for patients with cirrhosis of the liver and portal hypertension.[41] Lung surgery has on occasion improved the patient's condition but is usually not indicated because of the generalized nature of the pulmonary disease. Therapeutic bronchoscopy has also helped on rare occasions, especially in older children,[79] but because of the lack of marked improvement in many instances and the adverse reactions in younger patients it is generally contraindicated.

Postural drainage to help patients expectorate, administration of digitalis, if cardiac embar-

rassment is present, and other supportive measures may be of assistance.

Acute salt loss through the sweat should be suspected when patients with cystic fibrosis are admitted to the hospital vomiting and dehydrated.[60] Thirst is an unreliable symptom. Hyperpyrexia, cardiovascular collapse or coma present a real medical emergency, and the patient is in imminent danger of death unless prompt and vigorous measures are undertaken to restore the depleted water and electrolyte stores.

CONCLUSIONS

At first pancreatic deficiency was considered the basic defect in cystic fibrosis (Andersen in 1938, Blackfan and May in 1938, and Harper in 1938). Accordingly, most attention was devoted up to a few years ago to studying the metabolic effects of the lack of exocrine pancreatic secretions. It was then pointed out that a widespread defect of mucous secretions throughout the body might explain many of the symptoms and much of the pathology in this disorder, and the name "mucoviscidosis" or "mucosis" was suggested (Farber in 1944, Shwachman in 1951, and Bodian in 1952). With the demonstration of consistent involvement of the sweat glands and of the parotid glands it became evident that cystic fibrosis is in reality a generalized disease affecting many or perhaps all exocrine glands, mucus-producing and others (di Sant'Agnese 1953).

It is now recognized[11,13] that in cystic fibrosis there is variable involvement of the areas affected. At times incomplete forms of this generalized disturbance occur; pancreatic function may be normal, just as there are patients with virtually no respiratory symptoms, some in whom cirrhosis of the liver dominates the clinical picture and, finally, some[8,11,13] in whom high concentrations of chloride and sodium in sweat are the only abnormal findings. This suggests independence of involvement of these various organs, perhaps under genetic influence. It is possible, of course, that in all instances all susceptible areas are affected but to an extent too limited to give rise to clinical manifestations. Symptoms may appear later, indeed may remain in abeyance indefinitely.

Recent studies indicate that in fibrocystic disease three different defects in exocrine secretions are present that need to be explained: (1) An abnormality of mucus production, affording a reasonable explanation for the pancreatic, hepatic and pulmonary symptoms is probably present. This hypothesis has received important support from studies[48,49] which showed the presence of an abnormal mucoprotein in the duodenal content of patients with this disorder. (2) An abnormally high concentration of electrolytes occurs in eccrine sweat and mixed saliva. (3) An increased parotid secretory rate is present. These exocrine glands, different in function and in the products they elaborate, are thus affected in different ways.

The basic defect, whatever its nature, appears to be genetically transmitted. A likely hypothesis (Childs, 1955) is that which assumes a gene (or genes) which causes the fully manifest although variable disease in homozygotes and incomplete or no expression in heterozygotes. Probably a significant number of affected heterozygotes are present in the adult population.

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Combined Staff Clinic

Mechanisms of Edema Formation and Principles of Management

THESE are stenotyped reports of Combined Staff Clinics of the College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York. The clinics, designed to integrate basic mechanisms of disease with problems of diagnosis and treatment, are conducted under the auspices of the Department of Medicine. The reports are edited by Dr. Richard J. Cross.

DR. JOHN H. LARAGH: Edema is a clinical sign, a manifestation, not a cause of diseases of varied origin. In this discussion of edema I shall attempt to emphasize its constant features and the attributes which appear to be essential for its formation and maintenance, regardless of the specific underlying disease process. In this way I hope to highlight those characteristics which may be a common denominator, whether the patient be suffering from heart failure, cirrhosis of the liver with ascites, the nephrotic syndrome, or even a more localized disorder such as a swollen, thrombophlebitic leg.

To be sure, it has been popular practice in the past to separate the edema of heart disease from all other edemas. However, there is as yet no convincing evidence that the edema of heart failure is controlled by specific and unique forces which do not operate to govern the disposition of body fluid in all other situations.[1] And if, in this general discussion of edema, I refer chiefly to material gleaned from studies of congestive heart failure, it is only because much of our understanding of the mechanisms of edema has emerged from observations on this particular group of patients.

We are concerned herein with progressive edema formation which is characterized by an increase in the total amount of salt and water in the body and an associated gain in weight. We shall exclude from our discussion the pulmonary edema of acute left ventricular failure, which primarily involves a redistribution of fluid already present in the body.

When there is chronic, progressive accumulation of salt and water, there must be displacement of this accumulated fluid outside the vascular tree. This displacement is governed by the Starling principle,[2] which states that the exchange of fluid between the blood stream and the surrounding tissues is controlled by the balance between the colloid osmotic pressure and the hydrostatic pressure. According to this principle, edema might result from (1) a reduction in the serum protein concentration with a fall in effective oncotic pressure; (2) an increase in the venous pressure or (3) a change in the permeability of the capillaries. Other factors promoting edema formation are: (4) increased protein content of the edema fluid; (5) decreased local tissue tension and (6) obstruction of the lymphatic drainage. These factors may be present in varying degrees in clinical states of edema. Their presence to a greater or lesser degree would appear to be essential for the production of a state of progressive edema.

In cardiac failure it is increased venous pressure which tends to promote transudation. In cirrhosis of the liver intrahepatic venous congestion and hypoproteinemia are important factors. In the nephrotic syndrome the factors responsible for edema formation are more obscure but include hypoproteinemia as well as probable diffuse capillary damage. Edema from malnutrition also is related to these two latter factors.

In addition to these disturbances of local fluid exchange relationships of tissues, it is now well recognized that abnormal retention of sodium chloride by the kidney is an essential concomitant of the development of the edema of heart failure, of cirrhosis and of the nephrotic syndrome. Although there are fundamental differences in the primary mechanisms of edema formation in these disease states, in respect to sodium metabolism they are all similar, being

characterized by a tendency to abnormal renal retention of sodium chloride and water. Any discussion of the mechanisms of edema must therefore be concerned primarily with a consideration of the physiologic factors governing the

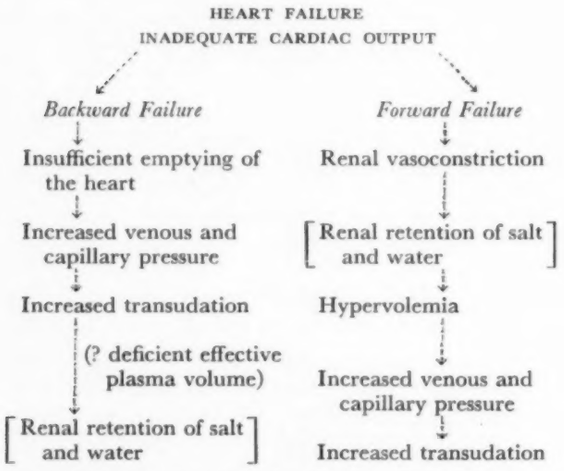

FIG. 1. Suggested mechanisms of heart failure.

disposition of ingested sodium chloride and water by the kidney in health and disease.

First, let us consider the hemodynamic factors involved in regulating renal retention of salt and water. Much of our knowledge in this regard has resulted from studies of patients with congestive heart failure. In Figure 1, I have indicated the two popular concepts of the mechanisms of congestive heart failure, the so-called backward failure and forward failure theories. The main difference between these two concepts is the dispute over the exact sequence in which the events occur. According to both theories, heart failure is initiated by an inadequate cardiac output; "inadequate" since, as we now know, the cardiac output may be quite low without evidence of congestive failure, and a high output is not necessarily associated with freedom from congestive failure.

The backward failure hypothesis postulates an initial elevation of venous pressure causing transudation. The renal retention of sodium and water is, in a sense, a reaction to this transudation. In the forward failure hypothesis, renal retention of salt and water is primary, leading to hydremia, to increased capillary pressure and then to transudation. This theory as originally proposed emphasized the glomerular filtration rate as a critical factor. Again, obviously common to both theories and, indeed, essential for the continued formation of edema fluid, is the abnormal retention of salt and water by the kidney.

For many years the importance of the kidney in this regard was not generally appreciated, and physiologists wandered far afield searching for an adequate explanation of dropsy. In this connection it is of interest that, although Starling is generally considered to be the father of the backward failure hypothesis, as long ago as 1896 he emphasized the importance of diminished excretion by the kidneys to explain the hydremia which then might lead to increased capillary pressure and transudation of heart failure. Nonetheless, as Starling's theory was subsequently evolved, his original mention of the kidney was apparently forgotten.

The backward failure hypothesis was held for a number of years. However, with the development of technics for the determination of cardiac output, blood volume and blood flow in various areas, certain inconsistencies became apparent. It was discovered that some patients in frank congestive failure had a normal or even increased cardiac output at rest. Certainly, here the heart did not seem to be failing as a pump, and yet there was increased venous pressure and edema. Furthermore, the blood volume of the decompensated patients was found to be increased fairly consistently, rather than decreased as some had predicted it should be if the edema fluid had been squeezed out of the capillaries.[1]

In 1940 Starr,[3] using a mechanical model, reported that increased venous pressure did not occur with failure of the pump unless the capacity of the circulatory system had been reduced or the blood volume increased. He also virtually destroyed the right ventricle of dogs without producing a rise in venous pressure. In addition, Starr noted that pressure in the heart and veins of cardiac patients immediately after death was some three times higher than in patients who had died from other causes. In death, there could be no backward pressure phenomena. He concluded from these observations that elevation of the venous pressure in heart failure was due to a combination of vasoconstriction in the kidney and hypervolemia produced by renal retention of salt and water.

As long ago as 1903 Widal had discovered that normal subjects gained weight when given large quantities of sodium chloride, and from this he concluded that the edema of renal disease was due to failure to eliminate salt.[4] He further de-

scribed diuresis with the excretion of 10 to 12 gm. of salt and a loss of weight of about 1.75 kg. within two or three days of the institution of a low-sodium diet in normal subjects, and a converse oliguria with the retention of salt and return to the control weight on re-adding salt to the diet.

It is fair to say that these observations were largely unappreciated for many years. In 1925 Gamble[5] observed that the administration of ammonium chloride did not cause a similar retention of salt and water and, in fact, produced a diuresis. From these data the obvious hypothesis was again advanced (this had also been previously suggested by the work of Pfeiffer, Magnus-Levy and Blum[6–8]) that the sodium ion was critical for the formation of edema. This concept did not gain wide acceptance for many years. In 1942 the full importance of sodium was re-emphasized by Futcher and Schroeder[9] who rediscovered that rigid restriction of dietary sodium results in a diuresis and loss of weight, and prevents reaccumulation of edema. They found further that the capacity to excrete a load of intravenous saline solution was greatly reduced in patients with heart failure as compared with normal subjects. The kidney was now incriminated, and the evaluation of its role in edema formation awaited the refinement and the application of physiologic technics.

In 1942 Seymour, who was not satisfied with the conventional explanation of passive congestion of the kidney as a cause of oliguria in heart failure, first studied kidney function in decompensated patients, using inulin and phenol red to measure glomerular filtration rate and renal plasma flow.[10] He discovered that filtration and plasma flow were both greatly reduced, plasma flow more than the filtration rate.

In 1944 Warren and Stead[11] began their study of heart failure by examining the edema fluid of decompensated patients. This fluid was found to contain small amounts of protein, less than 0.5 gm. per cent. This seemed to them to rule out lymphatic obstruction and increased capillary permeability as possible factors, the composition of the fluid seeming more compatible with hypoproteinemia or increased capillary pressure. The latter could be the result of an increased extracellular volume due to renal retention of sodium and water.

Hypoproteinemia is not a consistent finding in heart failure, and Warren and Stead set out to prove whether or not increased venous pressure was responsible. To do this, two patients with compensated failure were given added salt in their diet. It was noted that these patients gained weight and that their plasma volumes increased before there was any detectable rise in venous pressure. This was in agreement with Starr's previous observation in his mechanical model, and from this evolved the forward failure theory. These workers postulated primary renal retention of salt and water with elevated venous pressure as a secondary phenomenon. In support of their hypothesis they cited a number of other situations in which there was no correlation between height of the venous pressure and the degree of edema formation.

Following this study, Merrill in 1946 reported on quantitative studies of kidney function in patients who were decompensated at rest.[12] He showed that while the blood flow through the kidney may be reduced to as little as one-fifth of the normal, the cardiac output seldom falls below half of the normal. Thus the kidney, normally receiving 20 to 25 per cent of the cardiac output, in failure may receive only 7 to 8 per cent. This means that blood is diverted away from the kidney in heart failure. It was Merrill's conclusion that this took place in an effort to maintain effective blood flow to more sensitive organs.

These studies revealed that there was no correlation between venous pressure on the one hand, and filtration rate and plasma flow on the other. There was, however, a direct relationship between the cardiac output and renal plasma flow. This seemed to substantiate again the forward failure hypothesis—that the increased venous pressure was a secondary phenomenon.

Merrill had found that a glomerular filtration rate of about 70 to 80 cc. per minute seemed to be the critical level for the retention of sodium. Those patients whose filtration rate was above this level seldom required mercurials, while those below this level frequently needed diuretics. It was thought that tubular function remained relatively unaltered in heart failure and that the decreased load of sodium presented to the tubules by reduction in filtration allowed more nearly complete tubular reabsorption of the smaller load of sodium and water, leading to an expansion of the blood volume and ultimately to edema.

Subsequent work by many investigators has now established beyond any doubt that when all other variables are controlled, there is a linear

relationship between the amount of sodium filtered at the glomerulus and the amount appearing in the urine. Slight changes in the filtered load per minute, of the order of 1 per cent (which cannot be detected by our best technics) can, in time, profoundly alter net sodium excretion. This relationship is illustrated by a controlled experiment carried out by Thompson and Pitts. They placed a balloon in the aorta of dogs and produced graded reduction in glomerular filtration rate. In a number of animals sodium excretion fell simultaneously with the induced fall in filtration rate.[13]

However, despite the fact that the filtration rate was thus undeniably established as an important governor of salt and water excretion, certain clinical and subsequently certain experimental observations remained difficult to explain by the assumption that this was the sole regulating mechanism. For example, in the following the glomerular filtration rate (GFR) bears no correlation to sodium excretion: (1) GFR may be low in various diseases without edema (Bradley); (2) ascites may occur in patients with hepatic cirrhosis with normal filtration rates (Epstein); (3) GFR may be supernormal in patients with nephrosis forming edema and may be low during the diuretic phase (Janeway); (4) GFR may remain low with diuresis and may be increased during edema formation in patients with cardiac conditions (Farnsworth); (5) edema can be formed rapidly in dogs with very high filtration rates (Davis and Howell); (6) the salt-depleted dog, when given sodium chloride, retains it maximally as GFR rises (Frieden, 1952); (7) the hypophysectomized dog with very low GFR has a normal sodium balance (Surtshin and White, 1951).

Thus it was soon observed that in certain patients in heart failure the glomerular filtration rate was not low enough to account for their state of decompensation. At first this was explained by the suggestion that with any exertion glomerular filtration rate would fall below the so-called critical level and induce failure. However, subsequent controlled experiments have incontrovertibly established that the renal tubule may operate independently of the glomerulus. For example, Davis and Howell, studying ascitic dogs, demonstrated that with filtration rates of 150 per cent of normal, sodium retention may nonetheless remain maximal.[14,15]

Another interesting experiment is that reported by Frieden,[16] whose dogs had normal filtration rates after sodium depletion. These dogs were then given back the sodium which had been removed from them, and the glomerular filtration rate rose as the sodium was being restored. Yet practically none of the sodium appeared in the urine. Barger and his associates have shown that dogs with valvular lesions exhibit a tendency to markedly increased renal tubular retention of a salt load very early in the course of their disease at a time when they are still well compensated and still hemodynamically entirely normal. Indeed, this retention is perhaps the first measurable defect.[17] It appears not to be associated with a reduction in the glomerular filtration rate.

These studies establish the renal tubule as a second discrete regulator of sodium chloride and water balance. We have thus set the glomerulus and the tubule as the final two sites for this regulation.

It has often been remarked that the kidney should be complimented on its intrinsic intelligence; yet for precise control of the amount and concentration of water and electrolyte in the body as a whole one might reasonably surmise that the kidney would have to receive messages from other stations. Let us now consider the known factors which act to modify this function of the glomerulus and the tubule.

It would appear that the glomerular filtration rate may change from moment to moment because of alteration in blood pressure and because of constriction or dilatation of afferent and efferent glomerular arterioles. Baroreceptors are known to exist in the arterial tree, and a system of checks and balances is probably in constant operation to adjust to various demands of the circulation. The glomerulus may be considered to be the end receptor which can be adjusted to meet these hemodynamic changes.

The activity of the tubule, on the other hand, appears to be subject to modification chiefly by humoral agents. The hormones known to influence this tubular activity include the antidiuretic hormone of the neurohypophysis, which promotes water reabsorption, and the sodium-retaining steroids of the adrenal gland. Aldosterone is the most potent of these but hydrocortisone, corticosterone, desoxycorticosterone, compound S and probably certain estrogens can also promote tubular reabsorption of sodium chloride. Still other naturally occurring substances may also play a role. For example, noradrenalin, which is not considered to be

primarily concerned with electrolyte balance, may acutely modify sodium excretion. Smythe et al.[18] have shown that this may occur without any measurable change in filtration rate.

Both the glomerulus and the tubule appear to function adequately without neurogenic control, and no significant defects have yet been noted in the function of denervated or transplanted kidneys.

One may now properly inquire, how does the kidney operate to preserve the steady state of body salt and water in normal subjects, and what are the mechanisms whereby glomerular and tubular activity are altered in states of edema formation?

First, we all know that a change in the sodium intake affects the kidney. Perera and Blood[19] have shown that the kidney is able to cut off sodium excretion when sodium is removed from the diet. This response can occur without change in the serum sodium concentration, and the means by which it is mediated have not been clarified. As will be discussed later, the response may perhaps be triggered by a change in body fluid volume or by a shift in the relationships of sodium and potassium.

Let us consider some of the other factors that are known to modify the renal excretion of sodium in the normal subject. Venous congestion usually produces a tendency for sodium retention. These experiments, in general, have lent support to the so-called backward hypothesis. Some are summarized in Figure 2 in which some of the sites are shown where tourniquets have been applied to produce venous congestion. Bradley and Mudge demonstrated that a belt around the abdomen produced sodium retention, and since then many other investigators, working on all parts of the body, have demonstrated that occlusion of the venous drainage invariably modifies sodium excretion. Blake and Wegria produced acute retention of sodium by inducing a specific increase in the renal venous pressure. Some of these workers reported changes in the glomerular filtration rate; others found no change. Again, as Dr. Gilman will subsequently show important changes in filtration rate may escape detection.

Thus it appears that when the effective circulation is reduced by a trapping tourniquet or by quiet standing, tilting or venesection, the kidney acts to restore the effective circulating volume by retaining the necessary aliquot of sodium and water. It is not certain, however, whether these acute responses are hormonally or hemodynamically mediated to the kidney. Some of these experiments may be interpreted as supporting the so-called volume receptor concept. This concept emphasizes the extraordinary tendency of the body to sustain the amount of fluid

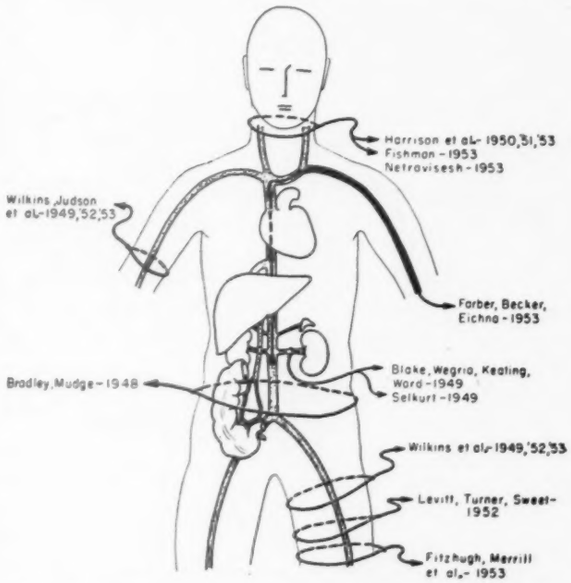

FIG. 2. Acute venous congestion and edema formation.

bathing and within the cells, at times at the expense of osmolarity of the body fluid. This effect is said by some to be mediated by adrenal or neurohypophysial activity, or both.

Of interest in this connection are experiments by Strauss.[20] He injected hypotonic solutions intravenously into recumbent subjects and observed increased sodium excretion, despite a falling concentration of serum sodium. This did not occur in the seated subject. He postulated a receptor in the cephalad portion of the body, sensitive to volume change. Indeed, Harrison and his group have reported sodium diuresis after cuffing the neck, and postulated a receptor in the head which modified sodium excretion. Other groups of workers have been unable to confirm this work, however.

Ever since Loeb[21] described the renal sodium and water loss and hemoconcentration of adrenal insufficiency in man, it has been appreciated that adrenal hormone activity might well act to promote sodium and water retention and thus maintain fluid volume. More recently, specific evidence has been presented, demonstrating that dehydration and overhydration may in

some way be an important stimulus for adrenal aldosterone secretion. However, no one has yet been able to isolate a specific volume meter from within the circulation, and it is not clear whether the sensitive volume involved is the total body

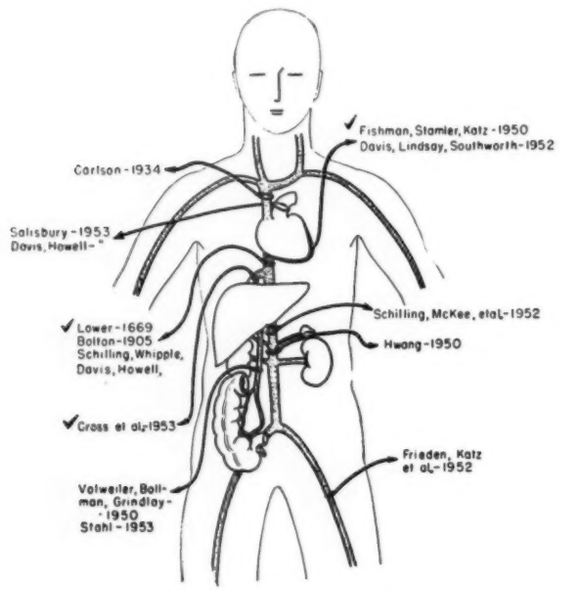

FIG. 3. Chronic congestion and edema formation.

water, the blood volume or the extracellular volume.

So much for this description of some of the acute adaptive responses of normal subjects. It is not known to what extent these phenomena are significant in chronic states of edema.

In Figure 3 is summarized what happens when more prolonged venous congestion is produced. When venous congestion is sustained, edema formation does not continue unless the constriction is placed in the four areas where the check mark has been made. In all other areas the transient sodium retention noted acutely gives way to a new equilibrium without progressive sodium retention. The data summarized suggest that hepatic congestion may be necessary, at least for experimental edema. At any rate, the venous pressure elevation must be present above the liver; even complete occlusion of the vena cava just above the kidneys does not, in the chronic experiment, lead to edema.

With this diagram as a starting point, I should now like to focus more closely on the hormonal factors in edema. As pointed out in the discussion of the two classic theories of heart failure, neither increased venous back pressure nor reduction in glomerular filtration rate always leads to sodium retention. What, then, do we know about the problem of hormonal control of the kidney?

Let us consider first the antidiuretic hormone of the neurohypophysis, vasopressin, which acts to promote water reabsorption by the tubule. Many workers have reported increased antidiuretic activity in the urine of patients with edema of all types,[22–24] especially the ascites of cirrhosis of the liver, and some have postulated a causal relationship. Shorr believed that VDM,* released by the anoxic liver, might account for the oliguria and water retention of edema by stimulating secretion of the antidiuretic hormone. In addition, it is not wholly clear whether sodium retention or water retention occurs first, although most of the evidence points to sodium retention as primary.

To study these problems we have utilized a preparation which, I believe, fulfills the requirements sought by Starling in 1910. Using an inflatable cuff developed by Dr. Jacobson of our surgical department, we can, in the conscious animal, make controlled observations during the onset and clearing of edema. The clamp is placed on the thoracic vena cava, above the liver. With occlusion of this vessel an immediate and sustained state of maximal sodium retention and massive, progressive edema occurs.

Figure 4 illustrates what happens when this vessel is occluded. The urine sodium content falls precipitously from above 100 mEq. per twenty-four hours to practically zero, and this is associated with the appearance of large amounts of ascitic fluid. Note also the striking, immediate natriuresis which occurs following release of the occlusion.

This technic was first applied to the study of dogs with diabetes insipidus. We soon found that massive, progressive edema could be formed in the almost complete absence of neurohypophyseal function, as confirmed by postmortem examination. The results of these studies are illustrated in Figure 5. One can see that the dog with diabetes insipidus retains sodium maximally, just as the normal dog does. The edema formation differs only in that it is characterized by persistent polydipsia and polyuria and urine of low specific gravity.[25]

This experiment has led us to assign a secondary role to the water-retaining hormone in the pathogenesis of edema. It remains entirely possi-

* Vaso-depressor material.

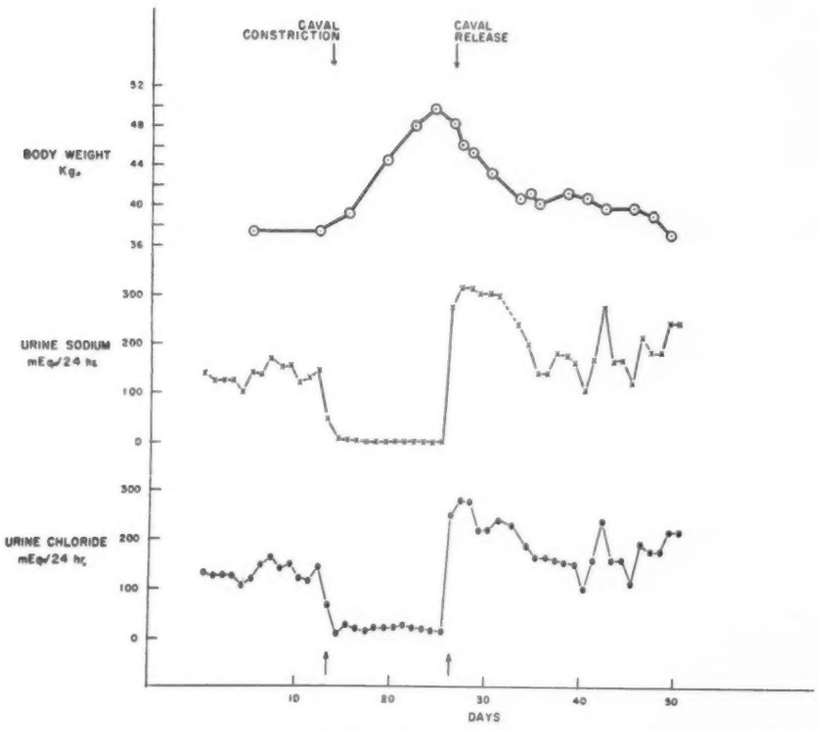

FIG. 4. Production of reversible edematous state in a dog on constant NaCl intake.

ble that overactivity of this hormone could aggravate edema formation by producing oliguria, retention of water in excess of salt, and hyponatremia. However, the experiment suggests again that the retention of sodium chloride rather than the retention of water is of primary importance.

Therefore, let us turn now to the sodium-retaining hormones. Some of the evidence that these hormones may be important has already been developed by inference. Additional clinical evidence is summarized as follows: (1) abnormal pattern of electrolytes of saliva, sweat, stool, urine (low sodium); (2) increased urinary excretion of steroids with salt-retaining activity can be correlated with edema and with degree of sodium retention; (3) diuresis of edema fluid may occur after bilateral adrenalectomy in the ascitic dog, in hypertensive and cirrhotic patients; (4) edematous states can be produced by overdosage of salt-retaining steroid (only limited edema in "normal" subjects, who may escape this influence).

Patients with edema exhibit an abnormal pattern of electrolytes in saliva, sweat, stool and urine. Indeed, they retain sodium in all areas. Again, this suggests a humoral effect.

Much of the credit for identifying this hormonal factor in edema must go to Luetscher who, with Deming, in 1950 reported that extracts of human urine, when injected into adrenalectomized rats, produced significant sodium retention in these animals. Luetscher continued his work, although at that time some investigators were willing to accept hydrocortisone as the sole, totipotent adrenal steroid. In a series of papers he reported consistently increased salt-retaining activity in the urine of patients forming edema from nephrosis, cirrhosis and cardiac failure.[26]

You probably all know the aldosterone story. This most powerful sodium-retaining hormone has been isolated from the adrenal glands, and the validity of Luetscher's original observations with crude urinary extracts has been amply confirmed by chemical technics. The new hormone may be secreted by the outer shell of the adrenal gland and is apparently not under anterior pituitary control. It has very little antiphlogistic activity.

According to certain presently available bioassay methods, normal adult human subjects excrete about 1 to 4 μg. of active hormone in twenty-four hours, and this may increase to about 6 to 20 μg. when such normal subjects are

given a low-sodium diet. Edematous patients on a low-salt diet may excrete much more; we have found as much as 600 μg. in one day's urine from a patient with congestive failure.

The role of the adrenal gland in edema formation is further illustrated clinically by the fact

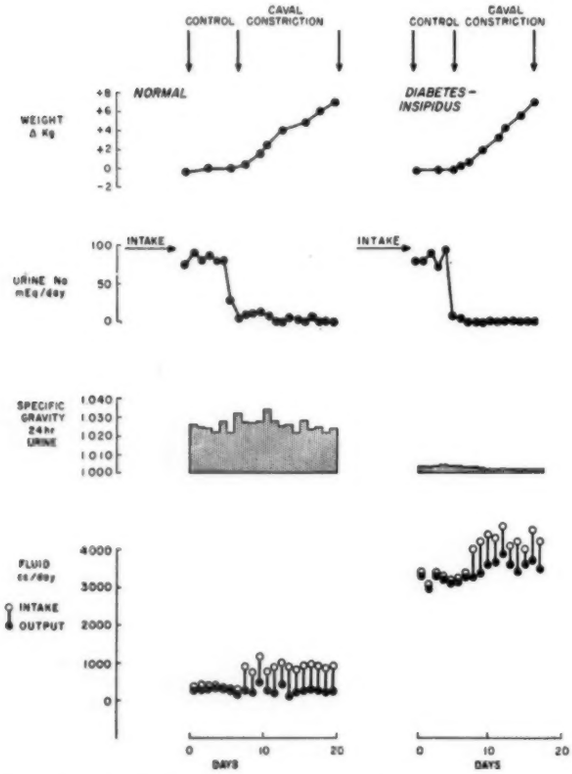

FIG. 5. Ascites formation compared in the normal dog and in the dog with diabetes insipidus.

that in certain patients with edema (hypertension, cirrhosis) diuresis occurs after adrenalectomy. However, the role of the adrenal gland is, perhaps, best illustrated by reviewing two important animal experiments, those of Davis et al. at Bethesda,[15] and of Stahl[27] in Strasbourg. Stahl ligated the portal vein of dogs and, despite an enormous increase in portal vein pressure, ascites did not occur. He then produced marked chronic hypoproteinemia with a carrot diet. Still ascites did not develop. When desoxycorticosterone (DOC) was then administered, ascites rapidly appeared.

Davis and Howell partially occluded the inferior vena cava above the liver and readily produced ascites of the type we have described in normal dogs. When the adrenal glands were then removed (Fig. 6), the ascites disappeared. The dogs could then be maintained edema-free on maintenance doses of DOC. As this dose was gradually increased, sodium retention and edema recurred in proportion to the increase in dosage. The results obtained with DOC probably are similar to those which would occur with

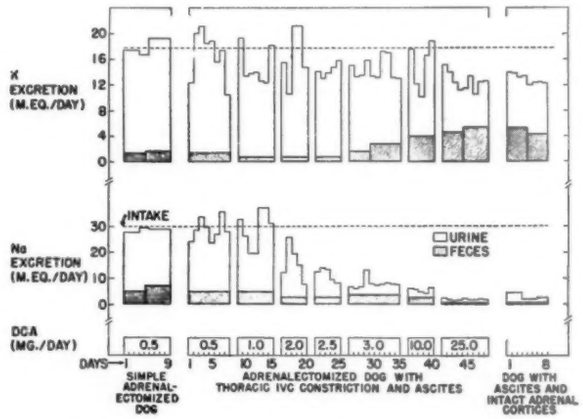

FIG. 6. Relationship of ascites formation to DOC administration.

aldosterone. In addition, we have now measured aldosterone in dogs forming edema after ligation of the vena cava and found that it rises promptly in the urine as soon as edema formation begins in these animals.

In view of these experimental findings, one might ask, can edema be produced in normal subjects with large doses of aldosterone? The answer is, usually not. Some sixteen years ago, in this department, a disease syndrome was first described in dogs given daily doses of desoxycorticosterone, by Kuhlman, Ragan, Ferrebee, Atchley and Loeb.[28] The disease was characterized by polydipsia, polyuria, weakness, periodic paralysis (especially of the hindlimbs), metabolic hypokalemic alkalosis and hypertension. This interesting syndrome was generally considered to be another example of species variation of no human significance. However, about a year ago, soon after aldosterone was discovered, Conn[29] reported similar symptoms in a thirty-four year old woman who had the same abnormalities of electrolyte metabolism. These symptoms were all completely cleared by removal of an adrenal tumor containing large amounts of aldosterone. The condition was called primary aldosteronism. Already it appears that it is not quite as rare as was originally thought, and several cases have now been reported. Of interest to us is the fact that these patients do not, as a rule, exhibit

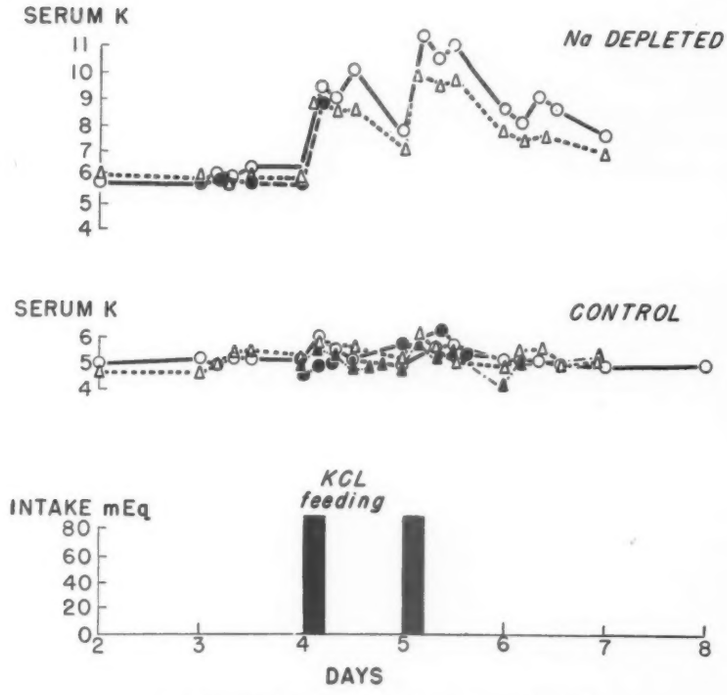

FIG. 7. Hyperkalemia related to sodium depletion.

edema but seem to reach an equilibrium between sodium intake and output; that is, they "escape" from the sodium retaining effect of the hormone.

The edemas are therefore examples of what one may call "secondary aldosteronism." It seems that some disturbance of the normal circulatory relationships must usually be present, in addition to the adrenal factor, in order for edema to develop. Apparently some other process must be in force, operating locally to disturb the integrity of the circulation and to promote transudation. However, it is fair to say that significant edema will not occur unless the adrenal is acting, and its activity (indeed, probably its overactivity) now appears to be more uniformly essential for edema formation than any of the previously described factors (that is, hypoproteinemia, venous back pressure, capillary damage, low glomerular filtration rate). Although adrenal activity is not primary, and heart failure obviously does not begin in the adrenal glands, under most circumstances there will not be much edema formation without adrenal activity.

Finally, having placed the adrenal in this very important light, one might ask, what is the stimulus for this adrenal activity, and why is adrenal overactivity so often apparent in these edematous states of diverse origin? This question cannot be adequately answered at present, but we do have some information. To begin with, it should be emphasized that the adrenal gland may not really be hypersecreting. Impaired hormone degradation, probably due to hepatic congestion, may be one critical factor in heart failure, in cirrhosis, and certainly in the experiments with dogs that have been described.

There has been considerable interest in the factors governing the rates of aldosterone secretion in normal subjects. The hormone appears to be largely devoid of anterior pituitary control. Luetscher showed that dietary restriction of sodium produces an increase in urinary excretion of the hormone. This occurs without change in the serum sodium concentration and the means by which it is mediated have not been clarified.

Could this regulator of the electrolyte environment be responding to some change in the electrolyte milieu? In the dog, we had observed a tendency for the serum potassium to be high under circumstances of sodium depletion.[30,31] This finding is illustrated in Figure 7. Thus the sodium depleted animal becomes exquisitely sensitive to the same dose of potassium chloride which previously hardly influenced his serum level of potassium. This relationship led us to suspect that the effect of the low salt diet, as

observed by Luetscher, might be mediated by potassium changes.[32] Figure 8 shows an experiment in a normal dog, which for the first eight days of the experiment had been fed a diet containing no sodium or potassium. Repeated analyses revealed that aldosterone was not

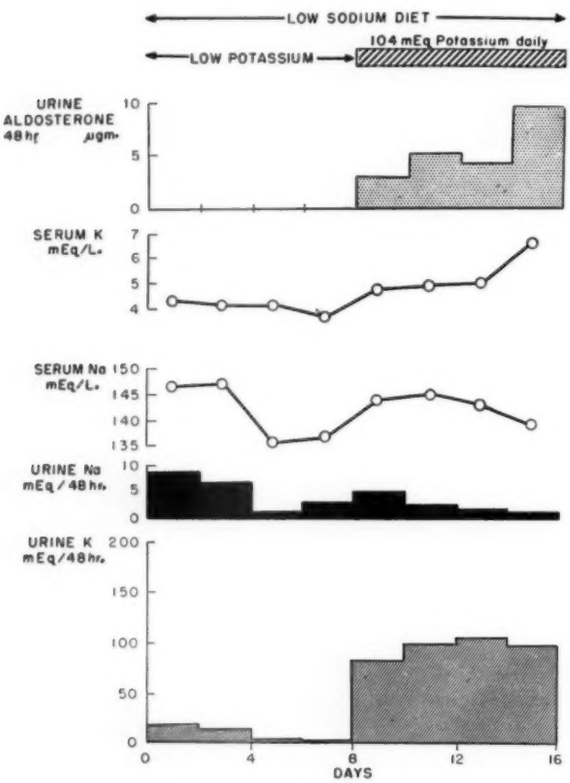

FIG. 8. Feeding experiment in a normal dog.

present in detectable amounts in the urine. However, when potassium was given in the diet relatively sustained hyperkalemia developed in the animal and, in association with this increase, aldosterone was found in appreciable amounts in the urine. Experiments of this type have led us to believe that, at least under certain circumstances, a rise in serum potassium may stimulate hormone secretion, and that the adrenal gland perhaps is directly sensitive to the potassium concentration of the circulating blood, acting to help restore normal sodium/potassium relationships in the body by promoting sodium retention and potassium excretion.

In addition, in a large number of dogs we could find no correlation between the serum sodium concentration (from 110 to 165 mEq./L.) and aldosterone excretion, whereas there was such a relationship between the serum potassium level and the urinary excretion of aldosterone.

On the other hand, Liddle et al.[33] believe that the aldosterone output may respond directly to changes in the volume of the fluid spaces. This concept is based upon the observation that the excretion of aldosterone tends to fluctuate inversely with the degree of hydration. It is possible that these two views can be reconciled. The potassium ion is intimately related to the state of intracellular hydration, and changes in volume may well be associated not only with change in pressure, but also with significant shifts of electrolyte and water which could well escape detection.

To what extent these stimuli are operating in disease is not known. In clinical edematous states there are no consistent abnormalities in the composition of body salts and water except for a general tendency towards reduction of osmolarity of body fluid. The picture is often confused by poor intake and by therapy. There is, furthermore, a paucity of information on the intracellular environment, where changes might be of more importance. In experimental edema, however, it is clear that hyperkalemia is a rather consistent finding. Most states of edema are associated with an increased total blood volume. Furthermore, in experimental ascites characterized by increased urinary aldosterone a sustained, large increase in plasma volume does not lead to sodium excretion as it does in the normal subject.

Thus the factors implicated in the secretion of aldosterone are sodium deprivation, potassium loading and reduction in body volume. From the evidence available to date, it appears that the striking activity of urinary aldosterone in edematous states cannot be wholly accounted for by any of these three possible mechanisms. The administration of sodium, the restriction of potassium or the rapid infusion of fluid, all reduce urinary aldosterone in edematous subjects but the values are still much elevated above normal.

Of course, the adrenal steroids which are under ACTH control, that is hydrocortisone (compound F) as well as compounds B and S and perhaps others, all have sodium-retaining activity and can, at times, tip the balance in the wrong direction. ACTH and cortisone have been observed to produce striking edema, even in presumably normal subjects. A similar tubular response may be observed with many other substances: phenylbutazone, licorice, puromycin, Rauwolfia, tridione® and certain estrogens.

The ultimate goal for the clinician in the management of edema is to be able to modulate this tubular activity and thus to prolong the asymptomatic phase of the disease state. We do not yet fully understand what stimulates the adrenal gland, and we have no idea how any of these sodium-retaining substances operate at the renal tubular level. When we do, perhaps we will then be able to develop an agent which will more fully control the reabsorption of sodium chloride by the renal tubules.

We now turn to Dr. Gilman, who will consider the problems of mobilization of edema fluid.

DR. ALFRED GILMAN: During recent years progress along three lines permits a more rational approach to the mobilization of edema fluid. First, there has been a better understanding of the pathologic physiology of edema formation, as presented to you by Dr. Laragh. Second, there has been a better understanding of the renal mechanisms of electrolyte excretion. Finally, there has been a better understanding of the mechanism of action of diuretic drugs.

As Dr. Laragh has indicated, no theory of edema formation can ignore the major role of the kidney, so let us turn our attention there and examine the renal events in somewhat greater detail.

It must be kept in mind that it is the work of the heart which produces the glomerular filtrate. In the normal, resting subject cardiac output is approximately 8,000 L. per day. Close to 25 per cent of this output goes to the kidneys. In other words, during a twenty-four-hour period the kidneys receive about 2,000 L. of blood, close to half of which is plasma. Approximately 18 per cent of this plasma is filtered through the glomerular capillaries (filtration fraction). Thus we produce about 180 L. of glomerular filtrate per day, or several times our body weight. It can be seen, therefore, that the formation of urine begins in a very prodigal manner.

The tubules now have to go to work and reclaim most of the constituents of the filtrate. How is this accomplished? Here we come to one of the most common misconceptions of renal physiology. Most capillary transudates return to the circulation by simple diffusion due to osmotic forces. However, the glomerular filtrate is delivered to the renal tubules where it comes into contact with an epithelial membrane. Tubular reabsorption is achieved by active transport,[34,35] a process which demands the expenditure of energy on the part of the renal tubular cells. This applies to both proximal and distal tubules.

There are many transport systems in the renal tubule. One that is very familiar is the transport system which operates in the reabsorption of glucose. It operates so efficiently that all glucose is removed from the glomerular filtrate, and the level of the glucose in the circulation has to be greatly elevated before glycosuria results. In other words, reabsorptive capacity greatly exceeds filtered load. However, we want to confine our attention to those transport systems which are involved in the reabsorption of extracellular electrolytes, primarily sodium chloride and sodium bicarbonate. How are these returned from the tubular urine to the extracellular fluid?

I want to digress for just a moment to speak of the principles of electroneutrality. If a positively charged ion is transported by the renal tubule, it must necessarily be accompanied by a negatively charged ion. Conversely, if a negatively charged ion is reabsorbed, it must be accompanied by a cation. It is not necessary to postulate a separate transport system for both ionic species. If chloride is reabsorbed, sodium would follow because of the electrostatic gradient that has been established. We cannot go into the evidence in very great detail but there is every reason to believe that the transport systems in the renal tubule are directed toward anionic reabsorption. As a matter of fact, to my mind it is almost axiomatic that transport is directed toward anions; otherwise, the kidney could never regulate acid-base metabolism.

Everyone talks very loosely about the reabsorption of sodium, but what would happen if renal transport were directed primarily toward the sodium ion? The anions would then follow and would be reabsorbed on the basis of their respective concentrations and mobilities. There would be no mechanism by which the kidney could regulate the chemical composition of the extracellular fluid, a function which it performs with such success.

Let us look at some of the reabsorptive mechanisms and observe to what extent primary anionic reabsorption can be implicated. Let us start with the reabsorption of bicarbonate. (Fig. 9.) You are familiar with the recent theories of the mechanism of bicarbonate reabsorption.[36,37] In the renal tubular cell there is a high concentration of carbonic anhydrase, the enzyme which catalyzes the hydration of CO_2. Thus the CO_2 which results from the metabolic activity of the renal tubular cells, or

that which is brought to the kidney by the circulation, is rapidly converted to carbonic acid. The carbonic acid then dissociates to yield H^+. The hydrogen ion from carbonic acid exchanges with sodium ion in the tubular urine, a process known as $H^+ - Na^+$ exchange or H^+

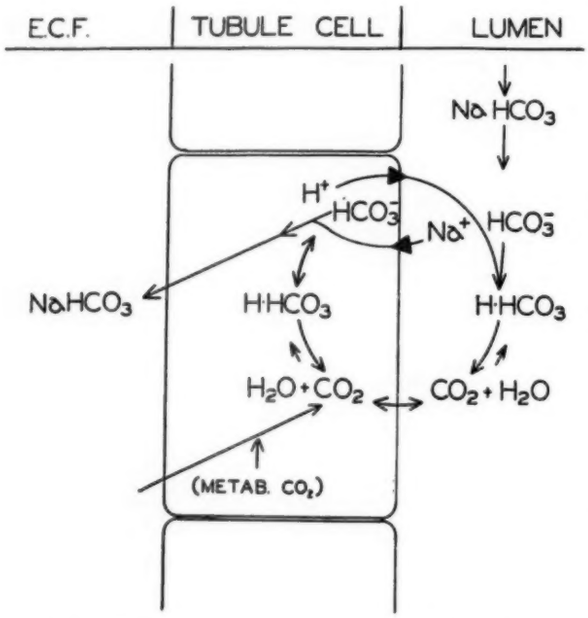

FIG. 9. The tubular reabsorption of bicarbonate.

secretion. As a result of the secretion of H^+, sodium bicarbonate in the tubular urine is converted to carbonic acid, which in turn breaks down to CO_2 and water. The Na^+ which exchanges with H^+ combines with HCO_3^- in the renal tubular cell and returns to the extracellular fluid as sodium bicarbonate. It is true that in this process the primary event is the transport of cation. However, it is a process designed to return fixed cation to the circulation accompanied by a specific anion, HCO_3^-. In this respect we can consider it as primary anionic reabsorption.

What about other anions? In the case of phosphate ion we know that there is a specific transport system and many of its characteristics have been defined. There is also a specific transport system for sulfate ion, although it has a very limited transport capacity.

This brings us to the chloride ion, the most abundant anion of the extracellular fluid. Is there a chloride transport system? I think, by analogy, there must be. Indeed, there is considerable indirect evidence for the existence of a specific chloride transport system. I want to show you an experiment that brings some evidence to bear on this problem. In the experiments depicted in Figure 10 the homeostasis of potassium was being studied.[38] Dogs were given a very low potassium diet for one week, and water per os to promote a water diuresis. The purpose was to produce a situation in which the homeostasis of potassium would demand complete reabsorption of the ion by the renal tubule. At the peak of water diuresis the dogs were excreting very little electrolyte in their urine and U_KV was practically zero. They were then given either large amounts of sodium chloride, a mercurial diuretic, or aminophylline. The purpose was to promote chloride excretion. If reabsorption of cation were the primary event in the renal tubule, there is no reason to believe that an increased excretion of chloride would result in increased excretion of potassium, since these animals had to preserve potassium to maintain homeostasis. However, if anionic reabsorption were the primary event, the production of a chloruresis should result in increased excretion of both sodium and potassium.

In Figure 10 attention should be directed to the solid circles which represent the data from animals on the low-potassium diet. It can be seen that, in response to the chloruresis, sodium excretion increased from very low levels to approximately 1,000 μEq. per minute. The rate of potassium excretion also increased. The solid line in the figure is not drawn to fit the data. Rather, it represents the ratio of the concentration of potassium to sodium in plasma. In other words, in response to chloruresis the excretion of both sodium and potassium was increased and the concentrations of these ions in the urine were in the same proportion as their concentrations in extracellular fluid. This is taken as evidence that the tubular reabsorption of chloride is primary and that monovalent cation follows passively.

Two important facts emerge from this concept. First, if there is no specific reabsorptive system for monovalent cation, one can appreciate the importance of the tubular secretion of K^+ in the regulation of potassium homeostasis. Second, an experiment of this type points out why, in any diuresis in which large amounts of the water and electrolyte are excreted, the patient will sustain a potassium loss.

With this brief background let us turn our attention to the kidney and see what the kidney normally does in order to maintain water and

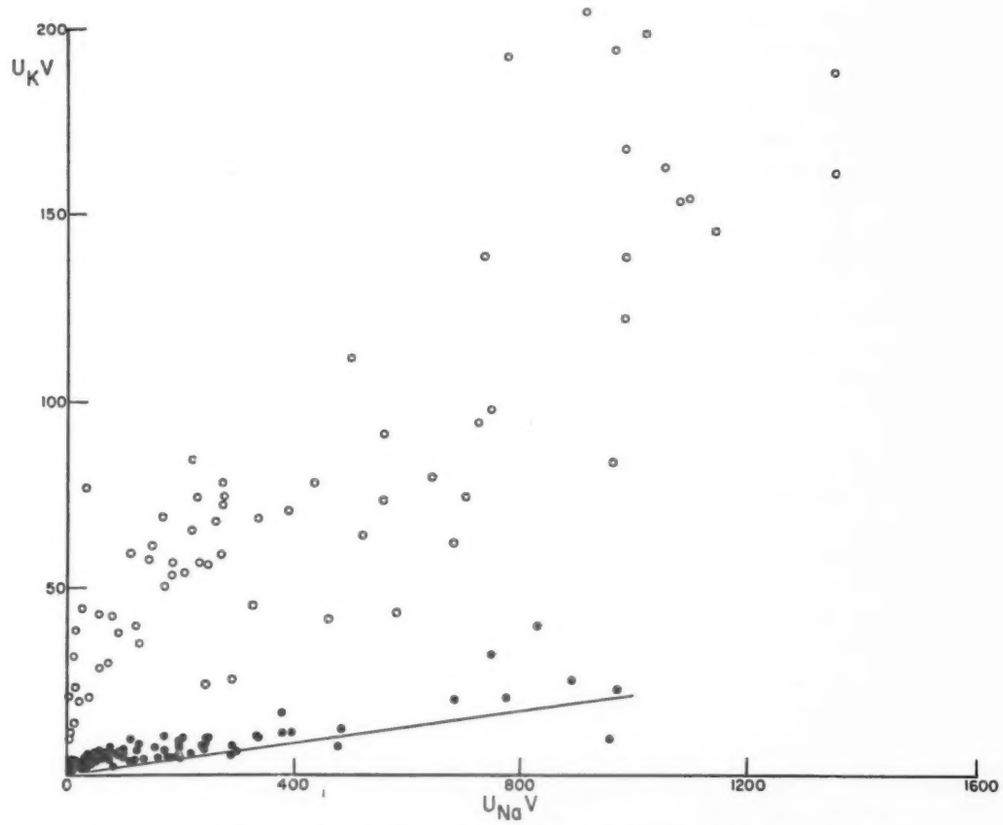

FIG. 10. The effect of chloruresis on the renal excretion of sodium and potassium.

electrolyte balance. Table 1 depicts the events that occur in a normal subject ingesting 10 gm. of sodium salts a day. In the daily glomerular filtrate of 180 L. there are approximately 1,600 gm. of sodium salts. The normal urine volume of such a subject would be approximately 1 L. which would contain the ingested 10 gm. of salt. To achieve this balance the renal tubules reabsorb 1,590 gm. of sodium salts and 179 L. of water. If this subject were to double his salt intake, he would also probably increase his water intake. The glomerular filtration rate and the composition of the extracellular fluid would show no appreciable change. In order to achieve balance, he would merely have to reduce the reabsorption of water to 178 L. and the reabsorption of sodium salts to 1,580 gm., a change of only a fraction of 1 per cent. It is obvious, therefore, that the reabsorptive capacity of the renal tubules is very great and a large amount of energy is being expended to return to the extracellular fluid compartment the large amounts of water and electrolyte which comprise the glomerular filtrate. It is also evident that the reabsorptive capacity of the kidney has to change very slightly to meet the exigencies of daily life.

Let us consider the situation in heart failure. (Table 1.) The person with heart failure has a very much reduced plasma flow, down from 1,000 to as little as 350 L. per day.[12,39] His glomerular filtration rate may not be proportionately reduced since, under these circumstances, filtration fraction rises. However, it may be as low as 120 L. per day. In this volume of glomerular filtrate there would be approximately 1,100 gm. of sodium salts. Thus the subject with heart failure would have to reduce his reabsorptive capacity to 1,090 gm. to achieve electrolyte balance with a salt intake of 10 gm. This is a far greater reduction than is ever demanded under ordinary circumstances, and it is doubtful whether or not homeostatic mechanisms exist whereby the transport capacity of the renal tubules can be reduced to this extent.

There is a second component to the edema of heart failure and other types of edema, as Dr. Laragh has indicated. Not only are the tubules presented with a greatly reduced load of sodium salts but there also appears to be increased

secretion of certain steroids which augment rather than decrease the capacity of the tubule to reabsorb electrolytes.

The abnormalities in renal function of the subject forming edema fluid may be described as glomerular-tubular imbalance. Glomerular-tubular imbalance is a condition in which there is such a discrepancy between filtered load and tubular reabsorptive capacity that the homeostatic functions of the kidney are compromised. In the situation under consideration, the filtered load has been so reduced and the reabsorptive capacity so increased that electrolyte reabsorption is complete.

TABLE I
BALANCE BETWEEN GLOMERULAR AND TUBULAR FUNCTION NECESSARY TO MAINTAIN EXTRACELLULAR ELECTROLYTE BALANCE

	Plasma Flow	Glomerular Filtration	Tubular Reabsorption	Urinary Excretion
Normal man, 10 gm. salt intake, 24 hours				
Water	1,000 L.	180 L.	179 L.	1 L.
Sodium salts		1,600 gm.	1,590 gm.	10 gm.
Normal man, 20 gm. salt intake, 24 hours				
Water	1,000 L.	180 L.	178 L.	2 L.
Sodium salts		1,600 gm.	1,580 gm.	20 gm.
Heart failure, 10 gm. salt intake, 24 hours				
Water	350 L.	120 L.	119 L.	1 L.
Sodium salts		1,100 gm.	(1,090 gm.)	(10 gm.)

How can one correct glomerular-tubular imbalance? First, the filtered load can be increased toward normal. That, of course, is the objective which has been achieved when a patient with a cardiac condition has been successfully digitalized, normal cardiac output and renal blood flow restored, and a diuresis induced. Secondly, one can try to bring the glomeruli and tubules into functional balance by depressing the renal capacity to reabsorb electrolytes. This is achieved by the use of diuretic drugs. The really effective diuretics are those which act by depressing the active reabsorptive transport of electrolytes by the renal tubules. Finally, there is a third device. If the kidney cannot excrete salt, one can achieve electrolyte balance by reducing electrolyte intake either by dietary means or by the use of resins. Time does not permit a discussion of all these methods of approach, so let us confine our attention to the depression of the renal tubular transport systems by drugs. Actually, we are going to limit our discussion to two types of diuretics, the mercurial diuretics and the inhibitors of carbonic anhydrase.

We have already mentioned the likelihood that the reabsorption of electrolytes in the renal tubules is directed toward the transport of anions. If a diuretic depresses a specific anion transport system, that particular ion will appear in abundance in the urine. Moreover, if the extracellular fluid sustains the loss of a particular anion, the composition of the extracellular fluid will be changed.

TABLE II
EFFECT OF MERCURIALS ON GLOMERULAR-TUBULAR BALANCE

Cl^-	100 mEq./L.
GFR	0.1 L./min.
Load	10 mEq./min.
Before Drug	
Reabsorptive capacity	10 mEq./min.
Excretion	0
After Mercurial	
Reabsorptive capacity	9.0 mEq./min.
Excretion	1.0 mEq./min.
Point of no return: Cl^- of 90 mEq./L.	

Let us now examine the mercurial diuretics in view of these considerations. There is every reason to believe that mercurial diuretics depress the reabsorption of chlorides. One of the best pieces of evidence is the composition of the urine which results from mercurial diuresis. The urine contains predominantly sodium chloride.[40] If the urine contains chloride as the predominant anion, and the source of this chloride is the extracellular fluid, it follows that as the volume of extracellular fluid decreases, the concentration of bicarbonate will rise and the concentration of chloride will fall. Metabolic alkalosis results.

Usually, the mercurials are given at intervals of several days. Their duration of action is measured in hours. During the interval there is time for renal compensation to occur. This is achieved by the renal excretion of a urine containing sodium bicarbonate.

What happens if mercurials are administered more intensively (Table II) and the opportunity

for renal compensation does not occur? Let us consider a person who has a chloride concentration in the extracellular fluid of 100 mEq./L. and a glomerular filtration rate of 0.1 L. per minute. The chloride load being delivered to the tubules (glomerular filtration rate times concentration) will be 10 mEq./minute. If this person has a tubular reabsorptive capacity of 10 mEq./minute, he will excrete no chloride. He will be in balance between the load and reabsorptive capacity. If a mercurial diuretic is now given and the reabsorptive capacity reduced from 10 to 9 mEq./minute, brisk diuresis would ensue. If successive doses of the diuretic were administered, allowing no time for renal compensation, the concentration of chloride in extracellular fluid would steadily fall until it reached a level of 90 mEq./L. At this point there would again be a balance between load and reabsorptive capacity. This person is now "refractory" to the mercurial diuretics. The kidney is not refractory. Tubular transport systems remain depressed. However, the load has been so reduced that a point of no return has been reached.

TABLE III
EFFECT OF DIAMOX ON GLOMERULAR-TUBULAR BALANCE

HCO_3^-	25 mEq./L.
GFR	0.1 L./min.
Load	2.5 mEq./min.
Before Drug	
Reabsorptive capacity	2.5 mEq./min.
Excretion	0
After Diamox	
Reabsorptive capacity	1.5 mEq./min.
Excretion	1.0 mEq./min.
Point of no return: HCO_3^- of 15 mEq./L.	

Let us look at the situation as it applies to a carbonic anhydrase inhibitor such as diamox.® (Table III.) Diamox inhibits the reabsorption of bicarbonate, and the urine excreted in response to diamox contains predominantly sodium bicarbonate. If bicarbonate is selectively removed from extracellular fluid, the concentration of chloride in extracellular fluid rises and that of bicarbonate falls. The end result is a metabolic acidosis. However, the duration of action of diamox is approximately six hours. If the drug is given once daily, there is sufficient time for renal compensation to prevent the development of persistent acidosis. However, what happens (Table III) if diamox is administered at intervals so frequent that renal compensation does not occur?

Let us consider a person who has a bicarbonate concentration in the extracellular fluid of 25 mEq./L. and a glomerular filtration rate of 0.1 L. per minute. The bicarbonate load being delivered to the tubules will be 2.5 mEq./minute. If this person has a tubular reabsorptive capacity of 2.5 mEq./minute, he will be in balance between load and reabsorptive capacity and will excrete no bicarbonate. If diamox is now given and the reabsorptive capacity reduced from 2.5 to 1.5 mEq./minute, a diuresis would ensue. However, if successive doses were given without opportunity for renal compensation, the extracellular bicarbonate concentration would fall to 15 mEq./L. At this point there would be a balance between the reduced load and reduced reabsorptive capacity. The point of no return has been reached.

What will happen with combined administration of these two diuretics? Diamox promotes the excretion of a urine which contains sodium bicarbonate and causes a rise in plasma chloride and a fall in plasma bicarbonate. Mercurial diuretics promote the excretion of a urine which contains sodium chloride and causes a rise in plasma bicarbonate and a fall in plasma chloride. Put the two together and a normal electrolyte pattern in extracellular fluid can be maintained.

I am not advocating that these two agents always be used together. However, the internist has the tools at his command whereby he can promote rapid mobilization of edema fluid and, at the same time, prevent the occurrence of distortions in the composition of the extracellular fluid. Thus if one wants to achieve a rapid mobilization of edema fluid without resorting to rest periods, the development of tolerance can be anticipated and can be avoided by controlling with appropriate drugs the anionic composition of the extracellular fluid. Many of you may say, "This is all very well from a theoretic point of view, but what about actual practice?" Many an internist says to me, "Diamox looks fine theoretically but practically we find it is not as effective acutely or in the long-term control of the edematous individual as mercurials."

I think there are two reasons why a carbonic anhydrase inhibitor is less effective than a mercurial for maintaining an edema-free state. In the first place, the load of bicarbonate delivered to the tubule is much less than that of chloride. If both transport systems were de-

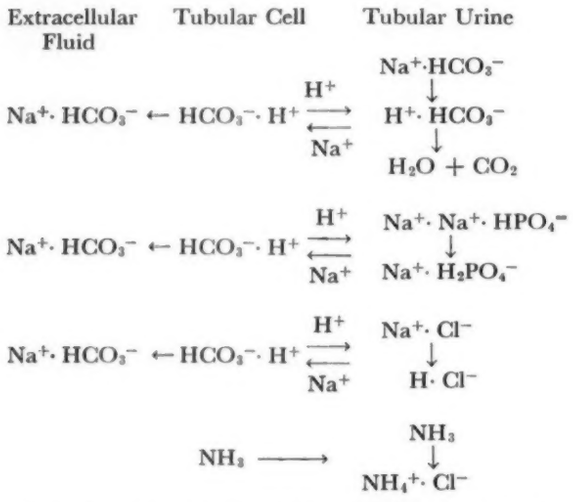

FIG. 11. Renal compensation for metabolic acidosis.

pressed proportionately, one would expect only one-quarter of the diuresis from the inhibition of bicarbonate transport that should be obtained from the inhibition of chloride transport.

However, there is another, more important explanation that has to do with the mechanism for compensation of the distortion of the composition of the extracellular fluid. If a metabolic acidosis is produced by the administration of diamox, compensation can theoretically be achieved in either of two ways. The renal excretion of sodium chloride and an isosmotic equivalent of water would both reduce the volume of extracellular fluid and restore its normal chemical composition. However, metabolic acidosis is a potent stimulus for the renal secretion of H^+. The H^+ secreted is derived from $H^+ \cdot HCO_3^-$ in the tubular cells. As the result of the exchange of H^+ in the renal tubular cell with Na^+ in the tubular urine, sodium bicarbonate is reabsorbed, as previously described. In addition, the fixed cation retrieved from the urinary buffers is returned to the extracellular fluid as $NaHCO_3$. Furthermore, by this process fixed cation can be retrieved from neutral salts, due to the renal synthesis of NH_3. This cation is also returned to the extracellular fluid in association with bicarbonate anion. These familiar events are depicted in Figure 11.[41,42]

In compensating for metabolic alkalosis the kidney does just the opposite. The secretion of H^+ is decreased. As a result, $NaHCO_3$ is incompletely reabsorbed and is excreted in the urine. Moreover, the urinary buffers are not acidified and are excreted with their full complement of fixed cation. The important point is that in compensating for metabolic acidosis the kidney conserves fixed cation and returns it to the body, a process that can augment extracellular fluid volume. In compensating for metabolic alkalosis the kidney excretes fixed cation thereby reducing extracellular volume. In other words, the compensation of a mercurial-induced alkalosis is in itself a fluid-mobilizing mechanism. However, this concept in no way detracts from the importance of the carbonic acid inhibitors in making a refractory patient already in alkalosis rapidly responsive to mercurials.

I do not want to leave you with the thought that the carbonic anhydrase inhibitors are the only drugs available to correct or prevent distortions in extracellular fluid from the intensive use of mercurial diuretics. You are all aware of the effects of ammonium chloride in potentiating mercurial diuresis. Let us give some thought to the mechanism. The following reactions are very familiar but their implications are often overlooked:

$$NH_4Cl \rightarrow \text{Urea} + HCl$$
$$HCl + NaHCO_3 \rightarrow NaCl + CO_2 + H_2O$$

The CO_2, derived from HCO_3^-, is excreted by the lungs. NH_4^+ is converted to urea. Although $NH_4^+ \cdot Cl^-$ is a highly ionized neutral salt, it does not add to the total fixed ion content of the extracellular fluid. Despite the fact that one is giving a cation, NH_4^+, and an anion, Cl^-, in the body a labile cation, NH_4^+, and a labile anion, HCO_3^-, disappear. The end result is a change in the composition of the extracellular fluid, namely, an increase in the concentration of Cl^- and a decrease in that of HCO_3^-. However, the volume of extracellular fluid remains unchanged since the total ionic content has not been increased.

As a result of the hyperchloremia produced by the administration of NH_4Cl, the filtered chloride load is increased. The depression of chloride transport by mercurial diuresis now results in greater excretion of water and electrolyte and more effective mobilization of edema

fluid. By the continuous concomitant administration of mercurials and NH_4Cl, the development of alkalosis and refractoriness can be prevented and maximal responsiveness maintained. Moreover, there is some evidence that acidosis *per se* may potentiate the action of mercurial diuretics.

Let me also remind you that cation exchange resins are also acidifying agents.[43] When a resin in the hydrogen or ammonium cycle exchanges with sodium in the intestinal tract, it not only binds and prevents the absorption of sodium but also forms HCl or NH_4Cl. The effect on the composition of the extracellular fluid is the same as that resulting from oral ingestion of these substances.

In conclusion, I would like to leave you with the following thought. I have limited my discussion to basic physiologic-pharmacologic concepts. However, they provide the foundation for rewarding therapeutic concepts. Today the physician has at his disposal more agents than ever before for the management of the patient with edema. For example, he has purified cardiac glycosides for the treatment of myocardial decompensation. He has a wide choice of diuretic drugs with different mechanisms of action. He has more efficient ways of restricting salt intake, with the advent of cation exchange resins, and the cooperation of the food manufacturers. But perhaps most important is the fact that our understanding of the pathologic physiology of edema formation, of the physiology of the kidney, and of the mechanism of action of diuretic drugs is rapidly expanding, and this provides the opportunity to correlate pathologic physiology with drug therapy. In the last analysis, this is the only way to devise a sound therapeutic regimen.

The physician who has a fixed, routine procedure for the management of cardiac edema, or any other type of edema, and who is remiss in determining the optimal regimen for an individual patient, is either endangering that patient or subjecting him to unnecessary discomfort and deprivation.

DR. LARAGH: Are there any questions?

STUDENT: I think you said that sodium depleted dogs tend to have a high serum potassium. What is the mechanism of this?

DR. LARAGH: Yes, it is certainly true that sodium depleted subjects exhibit a tendency to hyperkalemia—indeed, we have found that it can be quite hazardous to administer potassium to salt depleted patients. Our balance studies in hyponatremic patients and in dogs suggest that there is no gross inability to excrete potassium. We are studying this phenomenon further but it seems probable that hyponatremic patients are unable to retain potassium within the cells.

DR. WILLIAM J. HENSLEY: I have never been quite clear about this glomerular-tubular imbalance. After all, there are many circumstances in which the filtration rate may be reduced to levels far lower than those seen in heart failure. For example, this occurs with vigorous excerise or in mild shock, yet these conditions do not lead to retention of salt and water. If reabsorptive capacity can be sufficiently reduced in these circumstances, why can it not similarly compensate in heart failure?

DR. GILMAN: With reference to the specific circumstances you mentioned, it should be pointed out that vigorous exercise is usually accompanied with a diminution in salt excretion, which is compensated for in rest periods. In shock there is usually a considerable amount of renal anoxia, which may interfere with tubular function. Finally, may I remind you of the many factors discussed by Dr. Laragh which alter the tubular reabsorption of salt and which thus may either correct or cause a glomerular-tubular imbalance.

DR. EDWIN P. MAYNARD: I wonder if Dr. Gilman would comment on other factors which may render a person refractory to mercurials. It seems to me we often encounter this problem in patients with normal serum chloride levels who have been given mercurials only intermittently.

DR. GILMAN: It is important to remember that the amount of salt presented to the tubules for reabsorption is not only a function of the serum level but also of the filtration rate. Progressive decreases in glomerular filtration may so diminish the load that even under the influence of mercurials the tubules can still reabsorb all the chloride in the filtrate.

SUMMARY

DR. RICHARD J. CROSS: Edema is a clinical sign encountered in a number of different diseases, but its progressive accumulation is always accompanied by certain physiologic changes. Recent research has shed considerable light on the vexing question of which of these changes contribute to, and which result from, edema formation. Local factors such as the permeability of the capillary wall and the interrelationships of

capillary, oncotic, tissue and lymphatic pressures are of considerable importance in many types of edema, particularly in determining the distribution of the fluid. But attempts to explain progressive fluid accumulation solely in terms of these local changes have failed to account for all the observed facts, and it has become increasingly apparent that hormonal and other factors affecting the kidney play a major role.

Since the salt and water appearing in the urine normally represents less than 1 per cent of that filtered by the glomerulus, it is evident that very small percentile changes in glomerular filtration may cause great alterations in fluid and electrolyte excretion, if tubular reabsorption remains unchanged. But there is good evidence that, at least under certain circumstances, the renal tubules show an amazing capacity to adapt to striking changes in glomerular filtration. The reabsorption of salt and water by the renal tubule is influenced by many hormones, but the most important of these would seem to be aldosterone. Increased amounts of this hormone are almost invariably present in the urine of edematous patients or of animals in which progressive edema formation has been induced. It is not yet clear whether this results from increased production of the hormone by the adrenal gland or whether it reflects decreased destruction by the congested liver.

The formation of edema can theoretically be controlled by (1) increasing glomerular filtration, (2) reducing tubular reabsorption, or (3) limiting the dietary intake of sodium. The second of these measures can best be attained by the use of certain agents which interfere with the tubular reabsorption of particular anions. Sodium, the most abundant cation, accompanies these anions into the urine, and diuresis ensues. Thus mercurials apparently act by diminishing the reabsorption of chloride ions. Prolonged administration of this agent tends to produce a hypochloremic alkalosis and diminished responsiveness to the drug. Diamox, on the other hand, is effective as an inhibitor of carbonic anhydrase, thus interfering with the reabsorption of bicarbonate ions. Its continuous administration tends to cause a metabolic acidosis. By proper combination of these two diuretics the internist should be able to obtain prompt diuresis without disturbing the patient's acid-base balance. Many other drugs are also available to meet particular requirements but their intelligent use requires an understanding of the physiology of edema formation and of the mechanisms of action of diuretic agents.

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Clinico-pathologic Conference

Hepatomegaly, Splenomegaly and Purpura

STENOGRAPHIC reports, edited by Amoz I. Chernoff, M.D., and W. Stanley Hartroft, M.D., of weekly clinico-pathologic conferences held in the Barnes and Wohl Hospitals are published in each issue of the Journal. These conferences are participated in jointly by members of the Departments of Internal Medicine and Pathology of the Washington University School of Medicine and by Junior and Senior medical students.

THE patient (No. 264404), a fifty-four year old white hotel owner, was admitted to Barnes Hospital for the first time on December 12, 1955, and died on December 28, 1955. The chief complaints were "liver trouble" and bleeding of five months' duration.

The patient had been essentially well until July of 1955, when he began having dull, inconstant pain in the left anterior portion of the chest and pain in the left upper quadrant of the abdomen which was unrelated to effort or position but sometimes was associated with ingestion of fatty foods. His appetite remained good. He began to feel weak and "run down." He had frequent severe night sweats but gave no history of chills or fever. Shortly after onset of these symptoms he was admitted to a hospital in his home town where a diagnosis of "liver trouble" was made and he was treated with iron, vitamins, gelusil® and hydro-bilein.® The symptoms remained unchanged and a month later he noted the onset of petechiae on his legs and three to four very dark, soft bowel movements per day. He was readmitted to a hospital where the platelet count was found to be 3,000 per cu. mm. and a questionable abnormality was noted on proctoscopic examination. Roentgenograms of the upper and lower gastrointestinal tract were said to show no abnormality. A retention of 24 per cent bromsulphalein after thirty minutes was noted. An alkaline phosphatase determination was at the upper limits of normal. The remaining liver function tests were said to have given normal results. Therapy consisting of prednisone, 10 mg. per day, was started. However, the petechiae became more widespread, the patient continued to have intermittent melena and often bled from the gums. During the five months prior to admission the patient gradually lost 19 pounds and the family doctor noted that his liver was gradually enlarging and was nodular. The patient denied jaundice, pruritis, nausea and vomiting and any noticeable lymph node enlargement.

On rare occasions the patient was known to have taken 6 bromoquinine capsules per day. Otherwise the past history, system review and family history were non-contributory.

Physical examination at the time of admission revealed the temperature to be 37.4°C., pulse 86 and regular, respirations 18, and blood pressure 120/80 (right arm supine). The patient appeared well developed, moderately obese and chronically ill. Ecchymoses of varying size and petechiae were scattered over the entire body and were most marked below the knees. One small, movable, firm, lymph node was noted in the left axilla. A recent episcleral hemorrhage was noted in the right eye as well as a small flame-shaped hemorrhage in the left retina. Old crusted blood was seen in the nares bilaterally, and blood blisters and palatal petechiae were noted in the mouth. The neck was supple. Examination of the lungs revealed that both diaphragms moved well and that the lung fields were clear to auscultation except for a patch of fine, moist inspiratory rales in the left mid-axillary region. Examination of the heart revealed the point of maximum impulse to be in the fifth intercostal space 2 cm. beyond the left mid-clavicular line. Heart sounds were normal and no murmurs were heard. The abdomen was somewhat obese but bulging in the left upper quadrant was apparent. In the left epigastrium could be felt a hard mass with a rough surface and smooth sharp edges that descended slightly with respiration; it was possible for the examiner to get his fingers beneath the edge of the mass. In the right upper quadrant of the abdomen an ill-defined mass was noted on which a sharp edge was not felt. This mass was somewhat softer than the mass in the left epigastrium. Although the spleen was not palpable, the area of dullness

to percussion over the splenic area was thought to be slightly enlarged. No dilated veins were noted on the anterior abdominal wall. The bowel sounds were normal. The genitalia were normal. Examination of the rectum revealed fair sphincter tone, no hemorrhoidal tags, no masses and a prostate gland that was moderately enlarged, firm and smooth. The extremities revealed 1 plus pitting edema of both ankles. The peripheral pulses felt normal. The neurologic examination revealed no abnormalities.

Laboratory data were as follows: hemoglobin 9 gm. per cent, red blood cell count 2.69 million per cu. mm., packed cell volume 29 per cent, mean corpuscular volume 108 cu. μ., mean corpuscular hemoglobin 33 gamma gamma, mean corpuscular hemoglobin concentration 31 per cent. Reticulocyte count was 21.8 per cent. Platelet count varied between 0 and 3,000 per cu. mm. White blood cell count 12,700 per cu. mm.; differential count: 13 bands, 80 segmented forms, 2 lymphocytes and 5 monocytes. There was 1 plus anisocytosis. Silicone clotting time was sixty minutes. No clot retraction was noted after twenty-four hours. Bleeding time (Ivy) was greater than 5 minutes. (The test was stopped because of the development of a hematoma.) Urinalysis revealed a specific gravity of 1.024, reaction 5.5, protein negative, sugar negative, microscopic 12 to 15 red blood cells per high power field. The stool was guaiac positive. Blood cardiolipin and direct Coombs' test were negative. Platelet agglutinins were demonstrated. Non-protein nitrogen was 31 mg. per cent, fasting blood sugar 82 mg. per cent, prothrombin time 100 per cent of normal, total protein 5.4 gm. per cent, albumin 3.8 gm. per cent, globulin 1.6 gm. per cent, cephalin cholesterol flocculation + −, thymol turbidity 4 units, van den Bergh 2.1 mg. per cent (0.8 mg. direct and 1.3 mg. indirect), acid phosphatase 0.9 units, alkaline phosphatase 7.4 units, serum cholesterol 237 mg. per cent, amylase 73 mg. per cent, uric acid 7.6 mg. per cent, calcium 9.8 mg. per cent, phosphorus 3.6 mg. per cent, sodium 138 mEq./L., potassium 4.2 mEq./L., carbon dioxide combining power 27 mEq./L., chloride 99 mEq./L. A "metastatic series" was interpreted by the radiologist as showing a soft tissue mass in the mid-upper portion of the abdomen, pneumonitis of the right lower lung, and hepatomegaly. Intravenous pyelograms were interpreted as being indeterminate for both renal collecting systems. Hepatosplenomegaly as well as hypertrophic osteoarthritis of the lumbar spine were demonstrated on the films. A gastric fill-up was done and was interpreted as showing extrinsic pressure causing deformity of the anterior gastric wall, probably due to enlargement of the liver. A barium enema was performed and the results were not entirely conclusive. A questionable constrictive narrowing was noted in the distal ascending colon which suggested the possibility of spasm, fecal material or constricting carcinoma. Diverticulosis of the descending and sigmoid colon were also seen.

Shortly after admission to the hospital the patient began to pass dark, bright colored blood through the rectum. Prednisone therapy was started using 40 mg. per day. On the third hospital day the dose was increased to 100 mg. per day. Following this increase in dosage rectal bleeding diminished markedly. A sigmoidoscopic examination failed to reveal either a mass or bleeding points. A biopsy specimen was taken of a more nodular-appearing ecchymotic area near the umbilicus, and the pathologic interpretation of this specimen was "purpura." Thrombocyte counts showed a very slight increase while the patient was on prednisone therapy and reached 37,000 per cu. mm. on the eleventh day of hospitalization. It was deemed advisable that a splenectomy be performed in order to control the bleeding. Consequently, on the fifteenth day of hospitalization an exploratory laparotomy and splenectomy were performed. Postoperatively the patient did poorly. Most of the time he was semi-comatose. The pulse and blood pressure were unstable, the latter frequently dropping to shock levels and responding to transfusions of whole blood. On examination the patient was found to have a distended, quiet abdomen with marked generalized tenderness over the abdominal wall and dullness in the flanks. The thrombocyte count following surgery rose to 104,000 per cu. mm. and following administration of 7 units of whole blood during and following surgery the hemoglobin rose from 9.4 gm. per cent to 11.4 gm. per cent. During the postoperative period oliguria was noted. Twenty-six hours after surgery the patient's blood pressure dropped to shock levels and did not respond to rapid infusion of whole blood. Shortly thereafter he died.

CLINICAL DISCUSSION

DR. EDWARD REINHARD: Dr. Powers, will you review the roentgenographic findings?

DR. WILLIAM POWERS: Roentgenograms of the chest were unremarkable, although the diaphragms were somewhat elevated. Some infiltration was noted in the right lung field which was interpreted as being due to compression rather than pneumonitis. The gastrointestinal series revealed the stomach to be normal. However, a large mass, believed to be an enlarged liver, displaced the stomach posteriorly and to the left. A smaller mass was noted to the left of the stomach which was believed to represent enlargement of the spleen. The pattern of the small intestine was normal. A barium enema was done which revealed multiple diverticula along the distal descending colon and sigmoid colon. The large bowel canalized to the region of the transverse colon, but canalization could not be carried further. The patient was allowed to evacuate and a repeat enema was attempted with canalization to the cecum. In the distal ascending colon, an area of narrowing suggested the possibility of carcinoma in this region. A repeat barium enema again revealed multiple diverticula in the descending colon as well as one diverticulum in the colon on the right side. The evacuation film did not demonstrate the area of narrowing as distinctly as on the previous examination, perhaps because this region of the colon may have been concealed behind the hepatic flexure. These observations were interpreted as representing possible carcinoma of the ascending colon. A repeat barium enema was suggested.

DR. REINHARD: The patient had moderately severe anemia with a reticulocyte count of 21.8 per cent. The mean corpuscular volume was increased as would be expected from the marked reticulocytosis. The mean corpuscular hemoglobin concentration was at the lower limit of normal rather than decreased, as would perhaps be expected, but this finding can probably be explained by the fact that the patient had received iron therapy for several months. While in the hospital the stools gave positive results to the guaiac test. The presence of guaiac positive stools, coupled with the history of black stools prior to institution of iron therapy, seem to establish that the patient had anemia that was due, in part at least, to blood loss.

Dr. Moyer, let us assume that the patient had a tumor somewhere in the abdomen or in the retroperitoneal area and that it was bleeding. Would you list in the order of frequency the types of tumors that would have to be considered on a purely statistical basis, disregarding the patient's symptoms and signs. What are the lesions arising in the abdomen that would commonly cause blood loss and iron deficiency anemia?

DR. CARL MOYER: Carcinoma of the stomach, first. Carcinoma of the colon, second.

DR. REINHARD: Among the several types of carcinoma that were considered in this case, carcinoma of the pancreas was regarded as a distinct possibility. Does bleeding sometimes occur from carcinoma of the pancreas?

DR. MOYER: Bleeding can occur in carcinoma of the pancreas after erosion into the duodenum, but that is very rare and usually occurs during the terminal period.

DR. REINHARD: Is the bleeding always due to erosion? In these patients does bleeding into the duct system ever occur?

DR. MOYER: No, I have never seen bleeding into the pancreatic duct.

DR. REINHARD: Does bleeding occur from carcinoma of the gallbladder?

DR. MOYER: With extreme rarity.

DR. REINHARD: For all practical purposes, therefore, the two main lesions to consider would be tumors of the stomach and colon. Is it true that in cases of carcinoma of the ascending colon bleeding is more apt to occur while in cases of carcinoma of the descending colon obstruction is more apt to occur?

DR. MOYER: In those on the left obstruction is more apt to occur. In those on the right side bleeding is more apt to occur, which produces anemia.

DR. REINHARD: Dr. Moore, as hematologists we are often confronted with patients who have hypochromic microcytic anemia, in whom no evidence of outward bleeding can be found, in whom the stools give a negative reaction to the guaiac test and in whom the gastrointestinal series gives negative results. Should pyelograms be obtained under such circumstances? In the absence of gross hematuria, can a patient lose enough blood through the kidneys to produce iron deficiency anemia?

DR. CARL V. MOORE: The answer to that question is an unequivocal yes. It is unusual, but I have seen at least two or three persons in whom a diagnosis of carcinoma of the kidney with ulceration was ultimately established, who were unaware of any hematuria and in whom no gross hematuria was detected during hospitalization. Nevertheless they had severe iron defi-

ciency anemia. One has to assume that usually only enough blood is lost to cause microscopic hematuria, but I have always suspected that episodes of greater bleeding went unrecognized.

DR. REINHARD: I have seen similar patients on at least two occasions. These persons ultimately proved to have renal cell carcinomas with hypochromic anemia as one of the first manifestations of the disease. Carcinoma of the left kidney was considered a possible diagnosis when the patient was first admitted to the hospital, but I believe that most observers thought that a tumor of the kidney was certainly a very unlikely diagnosis. Dr. Moyer, do you believe that, having taken all the data into consideration, we may make a fairly definite diagnosis of carcinoma of the colon?

DR. MOYER: No.

DR. REINHARD: Would you put this diagnosis down as your first choice?

DR. MOYER: Considering the history and physical findings, I would not. I would consider a retroperitoneal tumor or one of the pancreas as being more likely. However, some tumors of the colon are difficult to demonstrate with roentgenograms, especially carcinomas of the distal colon. Consequently, I believe a repeat barium enema was indicated.

DR. REINHARD: I would certainly agree that a repeat barium enema was indicated. If the patient had carcinoma of the ascending colon, it would be necessary to look elsewhere for an explanation of the pain in the left upper quadrant of the abdomen and the left lower chest. I would like to ask you another question, Dr. Moyer. If this patient did have a carcinoma of the ascending colon with metastatic disease of the liver, could the metastases account for the pain? How often does pain occur from metastases to the liver?

DR. MOYER: Severe pain is very rare.

DR. REINHARD: You would not wish, therefore, to attribute whatever symptoms the patient had to metastatic disease of the liver. You would be more inclined to ascribe this pain to a tumor of the pancreas or some other retroperitoneal tumor.

DR. MOYER: That is correct. The pancreas or other retroperitoneal structures.

DR. REINHARD: Would you tell us more about the type of pain complained of by a patient with carcinoma of the pancreas. Is this type of pain characteristic?

DR. MOYER: The pain of carcinoma of the pancreas is not characteristic, except that it occurs especially during the night hours, usually after two or three o'clock in the morning, and wakes the subject. During the day the person is often free from pain. For that reason and because of the usual vagueness of the physical signs, roughly one third of all persons with tumors of the upper portion of the abdomen are seen by psychiatrists and their problems treated as psychosomatic ones before the lesion is discovered.

DR. MOORE: Dr. Reinhard, I wonder if Dr. Moyer would amplify his statement about pain accompanying metastatic lesions to the liver. It is my impression that the type of person in whom pain is most likely to develop is one in whom metastases develop just below the surface of the liver so that Glisson's capsule is stretched. It was thought, from the physical examination, that this man had nodules just underneath the capsule.

DR. REINHARD: If the onset of the pain had occurred within just a month or so, would you then be willing to attribute the pain to stretching of the capsule of the liver?

DR. MOYER: If the pain had been on the right side of the lower chest or upper abdomen I would. But this was left-sided pain. I have not seen persons with massive involvement of the left lobe of the liver who had pain on the left side when the right lobe was normal in size.

DR. REINHARD: Dr. Shank, have you had any experience with patients in whom carcinoma of the left side of the liver had been proved and was accompanied by pain of this character?

DR. ROBERT SHANK: Pain can certainly occur in persons with metastatic carcinoma of the liver as well as in those with primary carcinoma of the liver. It is usually stated that pain does relate to lesions directly beneath the capsule.

DR. REINHARD: Dr. Shank, would you discuss the laboratory findings in terms of the probable type of disease of the liver. Do you think that hepatic function tests are most compatible with metastatic disease or with other types of disease of the liver. One of the outstanding laboratory findings in this patient was the marked retention of bromsulphalein.

DR. SHANK: It is my belief that these findings are most likely associated with some infiltrative process in the liver similar to that seen in metastatic carcinoma. The other tests of hepatic function, such as the cephalin cholesterol flocculation, gave negative results. Thymol turbidity

was normal. Little increase occurred in the bilirubin. With the evidence we have at hand, including the history, I would think the most likely process would be of the metastatic type.

DR. REINHARD: How much metastatic disease must be present in the liver to produce significant bilirubin retention? The level here does not represent significant retention.

DR. SHANK: The serum total bilirubin (van den Bergh) is 2.1 mg. per cent which is not as high a figure as can be reached. Ordinarily in terms of the disruption of this and other functions of the liver like the bromsulphalein test rather massive involvement by any kind of infiltrative process must occur, whether of a carcinomatous, tuberculous or lymphomatous nature.

DR. REINHARD: I would now like to consider one of the most interesting features of this case, the severe thrombocytopenia, which apparently appeared rather early in the illness. Dr. Jim, would you discuss the role of the platelet in hemostasis and explain more specifically the various mechanisms by which the platelets play an important part in clotting. What is the significance of the failure of the clot to retract in this patient?

DR. ROBERT JIM: Platelets have been shown to be concerned with almost every phase of normal blood coagulation. A quantitative reduction in platelets or qualitative changes in the presence of a normal platelet count may lead to abnormal blood coagulation. There are approximately eight different ways in which platelets insure normal hemostasis. Platelets may act as viscous bodies in mechanically plugging blood vessels that have been disrupted. Normal capillary integrity is dependent to a large extent upon the presence of adequate amounts of serotonin liberated by platelets. We are all familiar with the poor clot retraction observed in persons with thrombocytopenia or thrombasthenia which is apparently due to a deficiency of the specific clot retracting substance produced by platelets. In the first stage of blood coagulation platelets liberate a substance which combines with the plasma coagulation factors to form the active plasma thromboplastin. Platelets also aid in the conversion of prothrombin to thrombin and of fibrinogen to fibrin. Lastly, platelets have been shown to contain not only antiheparin substances but also to possess antifibrinolytic activity. Abnormalities in any one of these platelet functions, as isolated defects, in combination or totally, may result in clinical hemorrhagic manifestations.

DR. REINHARD: Dr. Moore, how often does severe thrombocytopenia occur in association with late neoplastic disease? Is thrombocytopenia very common?

DR. MOORE: No. I have only seen it on two occasions in persons with malignancies other than lymphomas.

DR. REINHARD: Is it not true thrombocytopenia can be associated with malignant disease if there is metastatic spread to the marrow and a leukemoid reaction?

DR. MOORE: If there are extensive marrow metastases of the malignant tumor, then thrombocytopenia may occur because of the replacement of marrow by tumor tissue. In my earlier statement I was referring to immune mechanisms in patients with malignancy as the cause of thrombocytopenia.

DR. REINHARD: The history states that on rare occasions this patient took quite a bit of bromoquinine. Does this drug ever affect the platelets?

DR. MOORE: Yes, in those who happen to be sensitive to quinine, but that is a rare complication of quinine therapy.

DR. REINHARD: Dr. Harrington, would you discuss the significance of platelet agglutinins in patients who have carcinoma. Would you also discuss the significance of agglutinins active against the platelets of this patient in contrast to those which react only with platelets from other persons. What is the role of multiple transfusions in inducing the agglutination phenomenon?

DR. WILLIAM HARRINGTON: How many transfusions did the patient receive prior to admission?

DR. REINHARD: I do not believe he had any.

DR. HARRINGTON: Approximately only 6 per cent of all persons have naturally occurring isoagglutinins for platelets. Accordingly, when the serum of a patient with thrombocytopenia is tested for antibody, using normal platelets for the antigenic material, the finding of platelet agglutinins in ninety-four out of a hundred instances presumably can be related to the disease. However, transfusions or in some instances pregnancies increase the incidence of isoagglutinins by invoking production of antibodies of the immune variety. Therefore, it is significant that the patient under discussion was not given transfusions prior to admission. Under the latter circumstances, when only isoag-

glutinins are present, the patient's platelets do not react with his serum; that is, autoagglutinins are absent. Our patient's serum was tested against his own platelets obtained after splenectomy and strong agglutination was noted. Accordingly, this man had autoagglutinins and they were undoubtedly responsible for the thrombocytopenia. The low platelet count responded favorably to splenectomy, a fairly characteristic feature of autoimmune thrombocytopenia. We have not made a systematic study of autoimmune thrombocytopenia in association with carcinoma, although we have studied in addition a case of immunologic thrombocytopenia and hemolytic anemia in a patient with carcinoma of the ovary. A few instances of thrombocytopenia occurring during the source of chronic lymphocytic leukemia have also been found to have an autoimmune basis.

DR. REINHARD: Any further comments, Dr. Chernoff?

DR. AMOZ I. CHERNOFF: For the sake of completeness we should mention as a possible diagnosis the disease thrombotic thrombocytopenic purpura. It is a little difficult to explain the 21 per cent reticulocyte response on the basis of blood loss alone, even in the presence of relatively massive bleeding. Actually there is little in the history and laboratory findings against thrombotic thrombocytopenic purpura. The patient had anemia which could have been hemolytic in nature. He had a leukemoid reaction and severe thrombocytopenia. No neurologic findings or signs were noted but, as we all know, neurologic manifestations may or may not be present. The complete picture of thrombotic purpura may not develop until very late in the disease. One may possibly explain the abdominal masses on the basis of large hematomas. These lesions are probably metastatic tumors, but for the sake of completeness I believe thrombotic purpura should be considered.

DR. REINHARD: Because the masses were stony hard, thrombotic purpura seemed to me an unlikely diagnosis. I was not aware that platelet agglutinins had ever been demonstrated in thrombotic thrombocytopenic purpura. Do you know whether that is true, Dr. Harrington?

DR. HARRINGTON: A number of attempts have been made to demonstrate platelet agglutinins, generally without success. Our own experience has been limited to four cases. In only one instance were we able to test the serum of this patient with his own platelets and obtain equivocal results.

DR. MOORE: May I make a comment as to why this man was subjected to a splenectomy. A biopsy specimen was taken from the mass around the umbilicus because we were anxious to get a tissue diagnosis if possible. When that procedure failed, it seemed that a diagnosis of malignancy had to be established before we could just give up since there were other possibilities, some of which Dr. Chernoff mentioned. The operation was actually an exploratory laparotomy. It was done in such a position that a splenectomy could be performed if nothing else were possible. The surgeon was able to establish a diagnosis on opening the abdomen, and he realized that he could not correct the primary situation. He felt however that he might at least correct the thrombocytopenia by taking out the spleen. The patient might have improved sufficiently to be comfortable for a little while longer.

DR. REINHARD: I would like to make a diagnosis of carcinoma of the ascending colon at the site suggested but not proved by the roentgen findings, with extensive metastases to the liver causing the abdominal mass and pain.

PATHOLOGIC DISCUSSION

DR. MALCOLM H. MCGAVRAN: This corpulent white man weighed 180 pounds at the time of autopsy. The skin and sclerae were moderately icteric. Numerous recent and resolving ecchymoses and petechiae were present over the arms, legs and back.

Twenty-seven centimeters from the anus in the sigmoid colon a mucosal lesion was seen which was 4 by 5 cm., licheniform, friable and ulcerated. (Fig. 1.) In the ascending colon no lesions were found except for a diverticulum. No anatomic data are available to explain the constriction seen radiographically in this site. Microscopically the sigmoid lesion is a relatively well differentiated adenocarcinoma. (Figs. 3 and 4.) The tumor has extended through the muscular coats of the bowel, involves adjacent mesenteric fat, invades veins and nerve sheaths, and partially to totally replaces a series of mesenteric, peripancreatic and porta hepatic lymph nodes.

The liver weighed 7,200 gm., measured 36 by 28 by 18 cm., and comprised the masses in both the right and left upper abdomen. Large, soft and centrally necrotic masses of metastatic tumor accounted for the marked hepatomegaly. (Fig. 2.) The spleen, removed at the time of

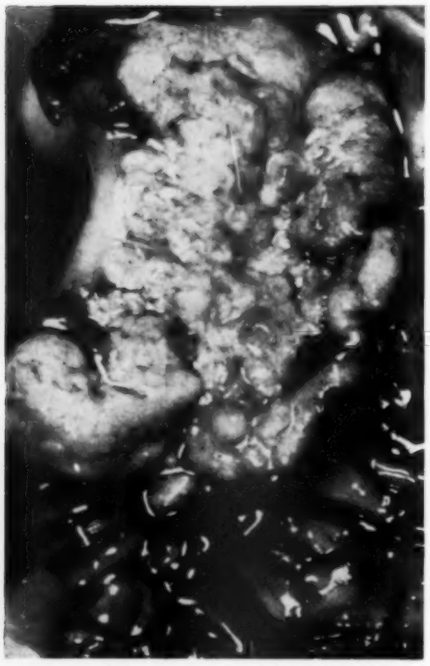

FIG. 1. Adenocarcinoma of the sigmoid colon, natural size. This is a relatively small and well demarcated tumor which is not concentric. The abrupt change in the appearance of the mucosa and the central ulceration are notable.

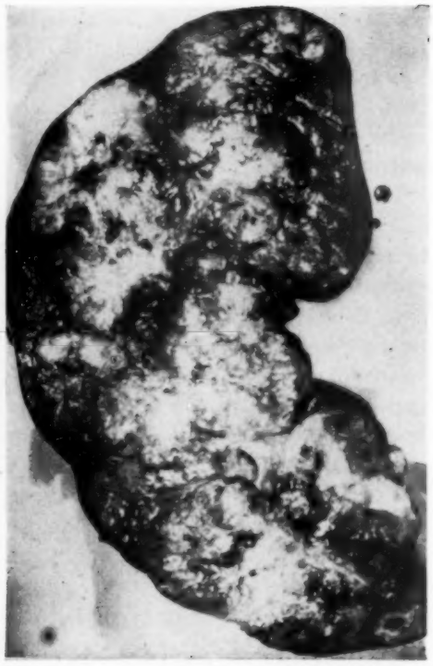

FIG. 2. Liver, reduced to one-fourth the natural size. All but a narrow margin of the liver is replaced by large umbilicated metastases. The adjacent liver is hemorrhagic in many areas.

exploratory laparotomy weighed 350 gm. and was slightly congested. A few small, 2 to 4 mm. nodules of metastatic tumor were found in the periphery of all lobes of the lungs. They were presumably of embolic origin via the hepatic veins, for no mediastinal lymph node metastases were demonstrable. Sections of the vertebral, costal and sternal marrow reveal a mild erythroid hyperplasia. Megarkaryocytes were present in usual numbers and no qualitative cytologic alterations were seen. No tumor was noted in any of the bone marrow sections.

Superficial erosions of the distal esophageal and gastric mucosa, with a half liter of altered blood in the stomach were among the agonal changes.

DR. WILBUR A. THOMAS: The anatomic features that have been described by Dr. McGavran are typical of adenocarcinoma of the colon. I believe that the massive involvement of the liver was sufficient to account for the symptoms related to the upper abdomen. We found no anatomic changes to explain the thrombocytopenia.

Final anatomic diagnoses: Adenocarcinoma of the sigmoid colon; metastatic adenocarcinoma involving the mesenteric, peripancreatic and portal hepatic lymph nodes, the liver (7,200 gm.) and the lungs; erythroid hyperplasia of the bone marrow; a five-month history of thrombocytopenia.

DISCUSSION AFTER PATHOLOGY PRESENTATION

DR. REINHARD: Dr. Harrington has done some special study on the tissues removed at autopsy and will discuss the findings.

DR. HARRINGTON: Some precedent has been established for suspecting that tumors of various kinds may induce production of autoantibodies for the formed elements in the blood. Thus it is well known that in some patients with malignancy, particularly lymphomas but also carcinomas, hemolytic anemia can develop with a positive reaction to the antiglobulin test. The recognition of autoimmune thrombocytopenia in patients with carcinoma is a more recent acquisition to our knowledge. The present case and the instance of carcinoma of the ovary already referred to raise certain questions regarding the relationship of carcinoma to autoantibody formation. In the patient who had carcinoma of the ovary we did not have an opportunity to attempt

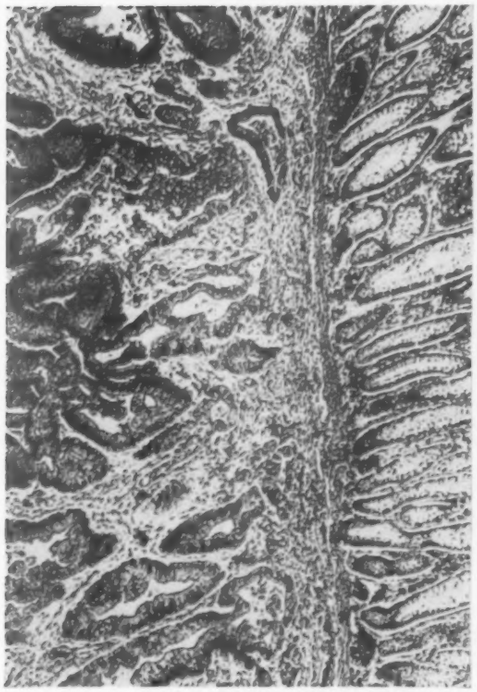

FIG. 3. Adenocarcinoma of the colon. Hematoxylin and eosin stain, × 27. The junction of the tumor with the orderly, well oriented glands and cells is shown.

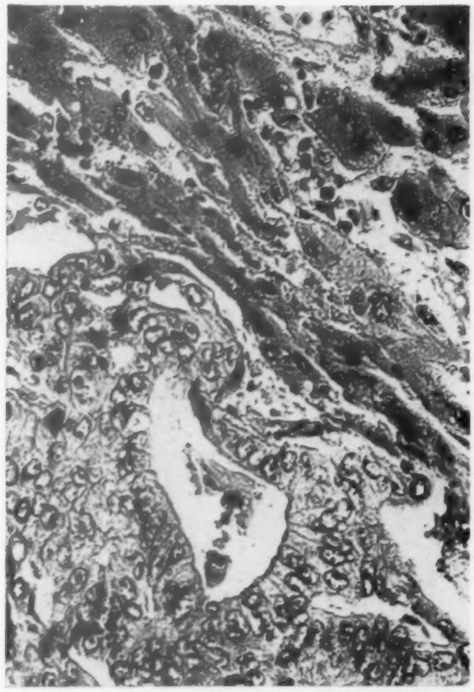

FIG. 4. Adenocarcinoma of the colon metastatic to the liver. Hematoxylin and eosin stain, × 150. Note the moderately well formed gland below with the surrounding disorderly tumor cells. Compression atrophy of the adjacent liver is seen above.

to absorb the antibodies in the serum with the tumor tissue. In the patient under discussion this opportunity was presented. When the serum was incubated with the primary tumor, the agglutinin was absorbed. Strangely, the intact normal bowel was equally effective in absorbing the platelet agglutinin. The metastatic, necrotic tumor in the liver seemed to be less capable of absorbing the antibody. The intact liver tissue did not remove the agglutinins. One can only speculate about the relationship of autoimmune hematologic disorders to malignant disease. The relationship may well be merely coincidental. It is more attractive to consider that the neoplastic tissue is foreign to the host and thereby antigenic; accordingly it may be capable of acting as a stimulus for the production of antibodies which may in rare instances cross react with antigens in the formed elements of the blood. One may ask whether or not splenectomy was justified in this patient. Dr. Moore recognized that this patient probably had metastatic carcinoma. The major problem was thrombocytopenia and associated bleeding. Nutrition was still good, and it seemed likely that the patient could be comfortable for some time if he didn't bleed to death. Unfortunately, however, he apparently died of liver failure.

DR. REINHARD: Dr. Moyer, had we known in advance that this patient had carcinoma somewhere in the colon with metastases predominantly to the left lobe of the liver, would this observation be grounds for believing that the tumor was to the left side of the colon? I have heard it said that metastatic disease in the left side of the colon is more apt to metastasize to the left lobe of the liver. It is difficult to understand why that should be so, since all metastases go via the same portal vein. Is there any truth to the statement?

DR. MOYER: I do not know. I have, however, heard the statement. The localization of the metastases is supposed to be related to the streaming effect in the portal blood flow. There are those who believe that there is something to this concept.

Acknowledgment: Illustrations were made by the Department of Pathology, Washington University School of Medicine.

Case Reports

Multiple Pulmonary Arteriovenous Fistulas in Juvenile Cirrhosis

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THE occurrence of multiple pulmonary arteriovenous fistulas in a patient with "juvenile cirrhosis," cyanosis, clubbing of the fingers and eventual cardiac failure served as the stimulus for this report. The patient was first seen at the age of eleven years and was followed over the next eleven years until his death. The clinical course was characterized by slow but progressive deterioration. The principal features of this case included hepatosplenomegaly and progressive liver failure, cardiomegaly and eventual cardiac failure. The latter was associated with a greatly elevated cardiac output, a diminished arterial oxygen saturation and the presence of a thoracic bruit. Clinical studies revealed (1) a very high cardiac output with no intracardiac shunt, (2) normal respiratory function, and (3) the presence of a physiologic intrapulmonary vascular shunt estimated at 40 per cent of the cardiac output. The shunt could not be identified on roentgenograms or by lung biopsy specimen secured at the time of thoracotomy. The most interesting observation at necropsy was in the lungs; by gross and microscopic examination no abnormalities could be found, but injection of the blood vessels of the right lung revealed the presence of numerous and widespread abnormal vascular channels. These channels were found to connect, directly and indirectly, the pulmonary arteries and veins. A review of the literature has not revealed reports of primary pulmonary vascular disease associated with cirrhosis of the liver.

CASE REPORT

The patient was first studied at the University of Minnesota Hospitals in August 1942, when he was eleven years of age. He was admitted to the pediatric service for evaluation of splenomegaly of marked degree. Birth, neonatal and developmental history revealed no abnormalities. The family history was non-contributory. During the year prior to admission the boy experienced intermittent jaundice of mild degree. There was no history of hematemesis or melena. Five months prior to admission enlarged tonsils and adenoids had been removed without ensuing complication. Physical examination revealed a small, pale, poorly nourished boy exhibiting retarded physical development. Marked abdominal enlargement, moderate ascites and distended superficial abdominal veins were noted. The superficial lymph nodes were slightly enlarged. The liver extended 9 cm. below the costal margin; the spleen was greatly enlarged and could be palpated 22 cm. below the costal margin in the midline. A soft systolic murmur was audible at the left sternal border. The chest appeared normal and x-ray examination did not reveal cardiac enlargement; the lung fields were normal. The blood pressure was 116/52. No telangiectases were visible in the skin or mucous membranes.

Studies of the peripheral blood and bone marrow revealed microcytic anemia and lymphocytosis without evidence of immaturity. The hemoglobin was 9.2 gm./100 ml., the erythrocyte count was 5.85 million/cu. mm.; leukocytes numbered 6,200 cells/cu. mm., with 49 per cent polymorphonuclear cells, 46 per cent lymphocytes, 2 per cent monocytes and 2 per cent eosinophils. Erythrocyte fragility, bleeding time, coagulation time and prothrombin time were all within normal limits. An evaluation of liver function showed normal values for serum bilirubin, urine urobilinogen and galactose tolerance but the cephalin-cholesterol flocculation test was 4 plus. The clinical impression was Banti's syndrome associated with hepatic disorder. Portal hypertension was assumed to be present, although esophageal varices could not be demonstrated by x-ray examination. Splenectomy

* From the Departments of Pathology and Medicine, University of Minnesota, School of Medicine, Minneapolis, Minnesota. This investigation was supported in part by a contract from the office of the Surgeon General, United States Army, Washington, D. C.

was recommended. The patient was advised to follow a high protein diet with vitamin supplements. He was admitted for a second time in October 1942, at which time an improved blood picture was noted. The ascites had disappeared. Operation was deferred for one year, during which time the patient was followed in the outpatient clinic.

In September 1943, the patient was admitted for splenectomy. This was delayed because a first degree heart block with prolonged P-R interval was noted in the electrocardiogram. Moderate cardiac enlargement involving both ventricles was apparent on x-ray, a change over that noted a year previously. Liver function tests revealed normal serum bilirubin, bromsulphalein retention of 7.5 per cent, and normal total and fractional serum proteins. The cephalin-cholesterol flocculation test was then 1 plus.

Splenectomy was performed in February 1943, despite persistent evidence of cardiac abnormality. The spleen measured 30 by 16 by 7 cm. and weighed 2,600 gm. The cut surface was deeply congested and dark purplish red. Microscopically, a large amount of blood was seen in the pulp; the corpuscles and trabeculae were very inconspicuous; some proliferation of the sinusoidal endothelium was present but there was no distinct fibrosis. Material fixed in Helly's solution showed large numbers of megakaryocytes. The liver at the time of splenectomy was lobulated and congested. A biopsy specimen of the liver revealed changes consistent with cirrhosis. Recovery from splenectomy was uneventful. Marked lymphocytosis was noted following splenectomy; the maximum leukocyte count, 32,500 with approximately 75 per cent lymphocytes, was recorded in October 1945.

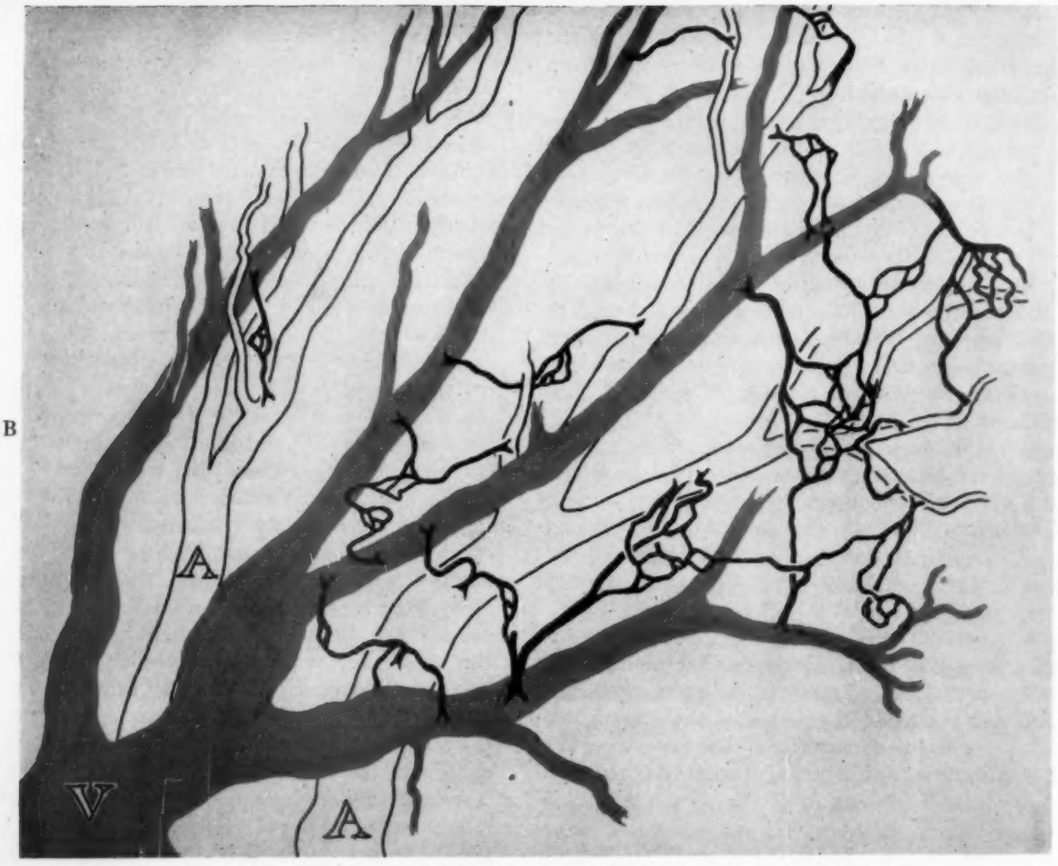
During the ensuing two years the patient was followed in the outpatient clinic. It was observed that the growth of pubic hair was markedly retarded and that the genitalia remained infantile. The liver during this period remained enlarged and felt smooth and firm to palpation. The blood pressure remained normal; the precordial systolic murmur previously detected appeared to increase in intensity. The patient first complained of exertional dyspnea in May 1945. Despite this the boy was able to lead a fairly normal existence and attended school. He experienced frequent infections of the upper respiratory tract. A year later, in September 1946, clubbing of the fingers and cyanosis were first noted. Exertional dyspnea increased so that by early 1947 the patient was severely incapacitated.

In August 1948, the patient was admitted to the Medical Service of the University of Minnesota Hospitals for evaluation of the cardiac status. Marked cyanosis was apparent. A few spider nevi were noted over the anterior and posterior upper portion of the chest. At this time the liver was enlarged to the level of the umbilicus. Examination of the chest revealed a moderate degree of emphysema; no rales were detected. No ascites or edema were present. The venous pressure and the circulation times were within normal limits. The patient's plasma volume was found to be 7.7 L. by the Evans blue dye method, a threefold increase over the expected normal value. The arterial oxygen saturation was 73 per cent and the venous oxygen saturation was 71 per cent. Marked cyanosis, which was present when the patient was at rest, largely disappeared when he was allowed to breathe 100 per cent oxygen. X-ray of the chest revealed cardiac enlargement with prominent pulmonary artery shadows. The peripheral vascular markings in the lungs were exaggerated and the emphysematous character of the lungs was confirmed. Films secured during the Valsalva maneuver did not reveal pulmonary arteriovenous abnormalities. The electrocardiogram still revealed a prolonged P-R interval but had otherwise not changed significantly.

The patient remained incapacitated throughout 1949; the frequent upper respiratory infections continued and one episode of pneumonia occurred. He was re-admitted in August 1949 and presented many of the physical abnormalities noted earlier. In addition, vision was found to be decreased in the left eye. Funduscopic examination revealed a "possible hemangioma" with suspected arteriovenous communications, but no aneurysms were seen. Marked retinal venous engorgement and distention was noted. A cluster of pulsating vessels, apparently veins, had developed over the right temple. For the first time, a systolic bruit accentuated on inspiration was heard over the right lung base. Cardiac catheterization was performed and a cardiac output of 20 L. per minute was found. No evidence of an intracardiac shunt was detected. The pulmonary artery and right ventricular pressures gave normal results. Angiocardiography failed to demonstrate any pulmonary arteriovenous communications nor did kymographic studies made over the entire chest. Measurements of pulmonary function (tidal air, vital capacity, residual air, functional air, total lung air) showed normal values. The carbon dioxide content of the whole blood was 19 mEq./L. In October 1949, a bilateral exploratory thoracotomy was performed. At operation no abnormalities were detected. A lung biopsy specimen taken from the left lower lobe was reported to show only slight emphysematous change. The vascular structures were not regarded as abnormal. The postoperative course was complicated by fever and a marked increase in jaundice, the serum bilirubin rising from a preoperative value of 2.8 mg. to 22 mg. per 100 ml. Antibiotics and digitalis were employed; the patient recovered slowly. On discharge from the hospital the serum bilirubin had fallen to 7 mg. per 100 ml.

The patient was seen at intervals in the outpatient clinic and admitted again in October 1950 for study. The outstanding physical features were cyanosis, mild icterus and clubbing of the fingers. The liver remained large. A few spider nevi were present. The other

1A



1B

(See legend on page 453.)

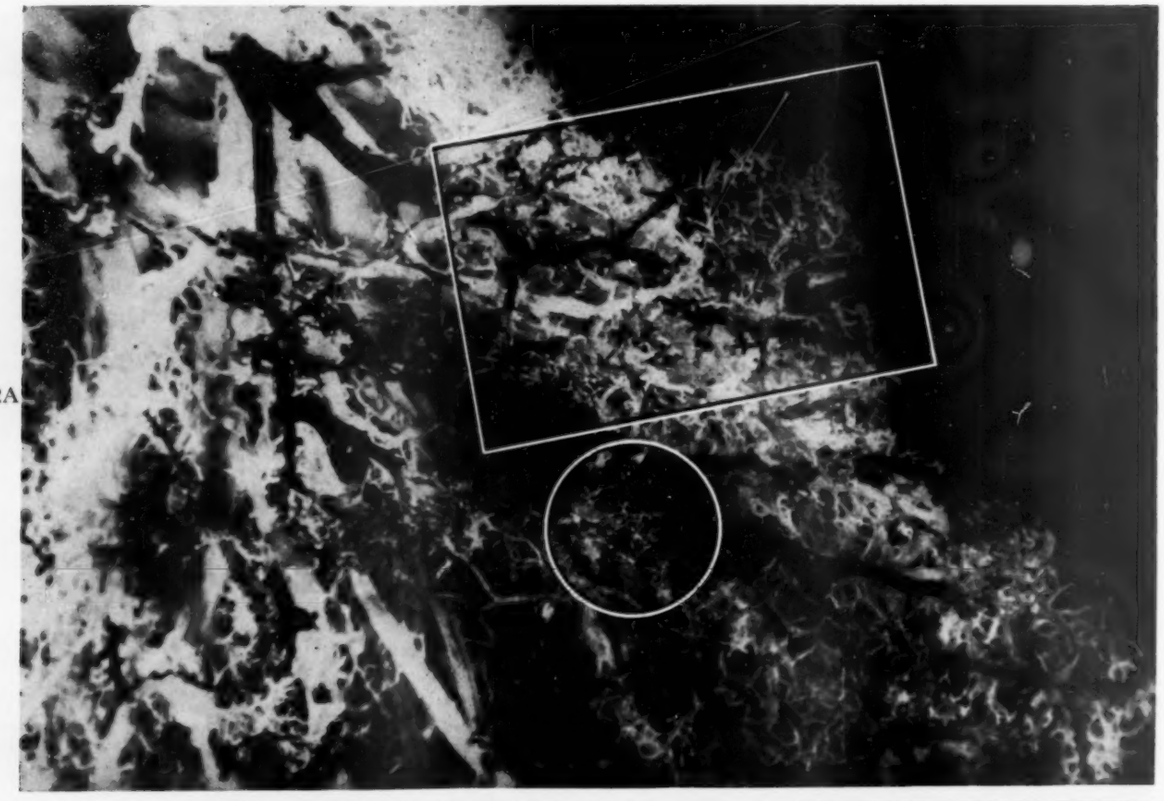
vascular abnormalities (temple and fundus) remained unchanged. At this time a diastolic bruit, as well as a systolic bruit, was audible over the right lung base. A similar finding was now noted over the left lung as well. Evidence of further impairment of liver function was demonstrable; the total serum bilirubin was 6.8 mg. per 100 ml. and the total serum protein measured 8.0 gm. per cent (albumin 2.6 gm., globulin 5.4 gm. per 100 ml.). A second cardiac catheterization revealed a cardiac output of 10.7 L. per minute; the mean pulmonary artery pressure was 8 mm. Hg. The arterial oxygen saturation was 72 per cent. Again there was no evidence whatever of an intracardiac shunt, either left to right or right to left. The right to left shunt (intrapulmonary), as calculated by the usual mixing equations, was 66 per cent. The Berggren technic[1] for calculating right to left shunts was applied. This technic involves the use of the polarograph for the direct determination. A wide difference was found between the partial pressure of oxygen in the patient's alveoli and that in the arterial blood. Application of the appropriate formulas showed that, by this technic, the patient had a 40 per cent right to left shunt. This latter figure was believed to reflect more accurately the actual size of the shunt. In view of these observations, in the absence of demonstrable pulmonary parenchymal disease, it was believed that the shunting was a diffuse vascular phenomenon and that the condition was not amenable to either surgical or medical correction. The patient was maintained on digitalis, a low salt diet and bed rest.

During subsequent hospital admissions the main concern was the symptomatic treatment of progressively deteriorating function of the heart and liver. The final hospital admission occurred in July 1953. Peripheral edema, severe dyspnea and orthopnea, marked venous distention and cardiac enlargement were outstanding features. The patient was deeply jaundiced. The total serum bilirubin was 47 mg. per cent. During the terminal period the blood urea nitrogen rose to 185 mg. per cent. Shortly before death the spider nevi and the pulsating lesion on the temple seemed to disappear, although the venous distention in general was prominent. The patient died on August 8, 1953.

Postmortem examination was performed three hours after death. The body was that of a poorly developed, extremely emaciated person who appeared to be much older than the actual age of twenty-two years. Marked jaundice, peripheral edema, severe cyanosis and clubbing of the fingers were evident. Hair on the head was abundant, but axillary and pubic hair was scanty. A few small, soft cervical nodes were palpable. The testes were small; both were in the scrotum. Distention of the neck veins and of the superficial abdominal veins was marked. The abdominal cavity contained two liters of a clear, straw colored fluid; the pleural cavities each contained 100 ml. of a similar fluid. The liver extended to 10 cm. below the right costal margin. It weighed 2100 gm., was dark green and exhibited the characteristic appearance of coarsely nodular cirrhosis. The nodules, varying greatly in size, measured up to 3 cm. in diameter. The fibrous tissue network compressed by the regenerative nodules was readily apparent since the latter were easily "shelled out." The gallbladder was small and the bile duct system was patent throughout. The kidneys each weighed 225 gm., they appeared uniformly enlarged with well defined architecture, normal calyces, pelves and ureters. The lungs were collapsed; both lung bases were firmly adherent to the diaphragmatic pleural surface. The right lung was removed and saved for injection studies. The left lung on cut section showed only collapsed, airless tissue. No abnormalities of the pulmonary or bronchial vessels were noted on direct inspection. The pericardial cavity contained a small amount of clear fluid; a fibrinous exudate covered the pericardial and myocardial surfaces. The heart was greatly enlarged, weighing 425 gm. A number of linear, vertical, calcified ridges were seen just above the mitral and aortic valves. The endocardium was normal except for the calcific deposits. The myocardium was smooth and without abnormality. The left ventricle was hypertrophied, measuring 1.5 cm. in thickness; the wall of the right ventricle measured 0.5 cm. in thickness. The coronary arteries were patent. All organs and tissues were bile-stained.

Microscopic sections of the liver disclosed a distorted lobular pattern with nodules of regenerated liver tissue surrounded by heavy bands of connecting tissue. The larger nodules had central veins and the interlobular connective tissue contained portal vessels

FIG. 1. A and B, the structures which show the abnormal shunting between the arterial and venous sides of the pulmonary circuit are black. The pulmonary arteries (A) and veins (V) of the injected specimen both appear light in this photograph because they contain the plastic injection mass that was injected entirely through the pulmonary arteries at the hilum of the lung. The vascular channels which did not show the abnormal communications have been dissected away. Figure 1B is a schematic diagram of the vascular structure as seen in Figure 1A. In the diagram, the vein is grey, the artery is outlined in black and the communications are solid black. The large vessel in the lower left corner is one of the large hilar pulmonary veins. One of the large pulmonary arteries can be seen below and to the right of this vein. Several small direct communications (black) can be seen connecting the artery and its large branches with the large pulmonary veins in the hilar region. In the right center portion of the photograph can be seen a complicated system of communications. This system is buried in the parenchymal portion of the lung; it is seen to be fed by at least one small vessel from each of the branches of the pulmonary vein visualized. This type of communication system is typical of the diffuse nature of the shunts and could be demonstrated throughout the parenchymal areas of the lung.



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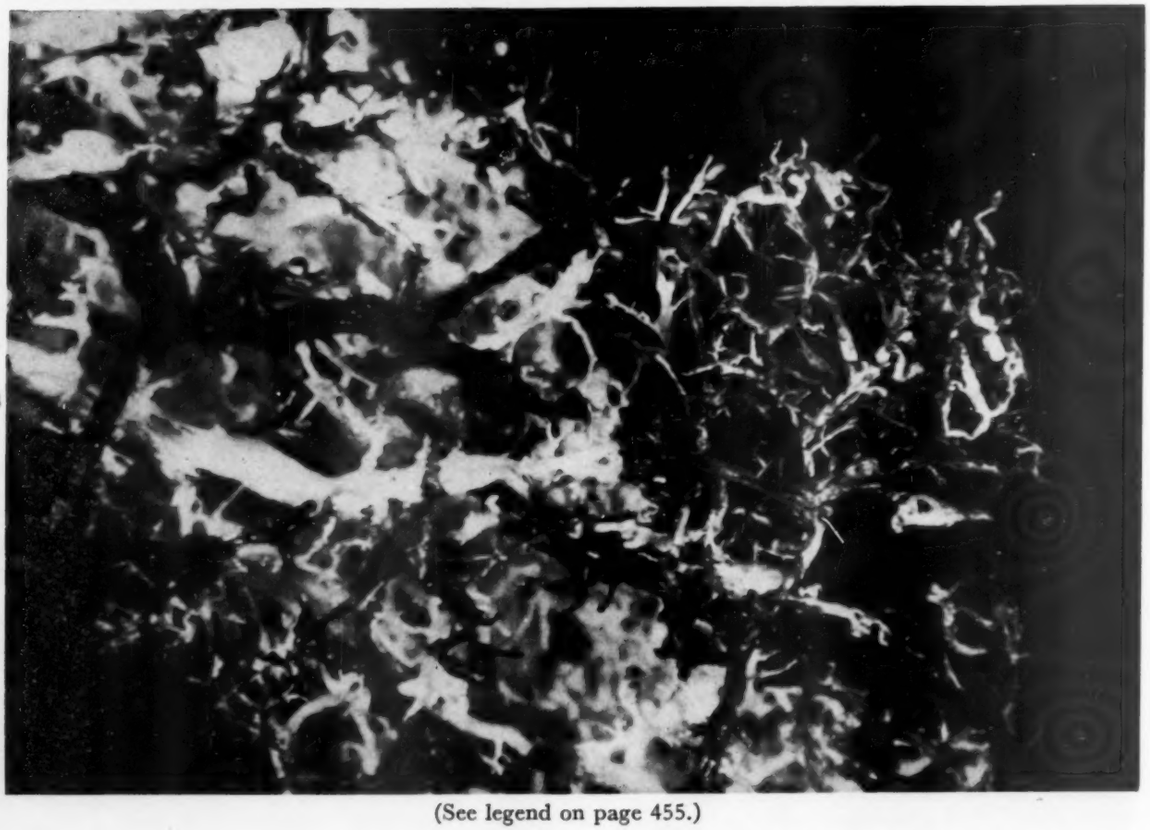

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(See legend on page 455.)

FIG. 3. Photograph of colored injection mass in vessels of middle lobe of right lung; coloring and reproduction as in Figures 2A and 2B. The area shows the direct type of communications found in subpleural locations. A branch of the pulmonary artery can be seen at the bottom of the photograph as the light vessels between the two black veins. The communicating channels appear grey and are seen to connect the arterial and venous channels.

and bile ducts. The smaller nodules, however, lacked any regularly placed vessels. Numerous small areas of necrosis of the liver cells were noted. Inspissated bile was seen in the radicles of the biliary tree. The changes described varied in severity from section to section, some portions of fairly normal liver tissue being seen. Large numbers of prominent subcapsular hepatic vessels were seen in all sections but no abnormal vascular communications could be demonstrated. The endocardial lesions, which have been described previously as calcific deposits, consisted of heavily calcified thick fibrous ridges, the significance of which is unknown; they did not represent vegetations. Sections of the kidney disclosed subacute glomerulonephritis. Bile-stained casts were noted in the tubules. Dilated submucosal veins, representing varicosities, were noted in the sections of the esophagus. The remainder of the gastrointestinal tract showed only edema and some vascular congestion. Hemorrhage into the perilobular spaces of the pancreas was present, with infiltration of neutrophils, edema and scattered areas of fat necrosis. The testes exhibited a marked decrease in spermatogenesis. The normal architecture of the lymph nodes was distorted; lymphocytic hyperplasia and fibrous tissue overgrowth was prominent. Examination of the brain gave entirely negative results. Microscopic examination of the lung revealed scattered areas of atelectasis; the parenchyma and the bronchi showed no particular abnormalities. The pulmonary vessels were prominent but single random sections did not exhibit any unusual vascular alterations or communications.

Immediately after removal of the right lung a solution of vinyl acetate (approximately 6 per cent in

FIG. 2. A, photograph of a colored injection mass in the middle lobe of the right lung. In this area the venous channels of the original preparation were black; arterial segments were left untouched and communicating channels were painted red. In this reproduction in black and white of the colored preparation, the arteries appear white, the veins black and the communicating channels grey. A portion of the injection cast has been removed in order to expose the shunts. An example of the complicated systems of communication found deep in the parenchyma of the lung can be seen in the lower central portion of the photograph (circle). B, an enlargement of the cluster of vessels seen in the square area in the upper right hand portion of Figure 2A. This mass of communicating vessels is supplied by several large arterial branches and drained by two veins.

acetone) was injected by syringe into the pulmonary artery. Although the pressure used during the injection of the plastic was not measured, the injection mass flowed freely under very slight pressure. Almost immediately the material escaped from the open pulmonary veins at the hilum. Therefore it was necessary to clamp these vessels so that the injection, accomplished entirely through the pulmonary artery, could continue. The total amount of plastic solution used was 300 cc. The specimen was placed in concentrated hydrochloric acid and the tissue macerated. The plastic network remaining was cleaned by washing with tap water. Study of the specimen revealed great numbers of abnormal vascular communications or shunts diffusely distributed throughout the entire vascular tree. Direct communications between the large pulmonary arteries and veins near the hilum were found. (Fig. 1A, 1B.) In addition, a complicated system of communications arising from several arteries and veins were found throughout the pulmonary vascular bed in a more peripheral location. (Figs. 1A, 1B, 2A and 2B.) Several large, directly communicating channels were found in subpleural locations. (Fig. 3.) The size of the abnormal communications between the pulmonary arteries and veins could not be accurately estimated by any direct measurement because of the shrinkage of the injection mass during hardening. (No filler was used.) The largest plastic cast considered to represent an abnormal channel measured 1 mm. in diameter. The majority were much smaller.

The anatomic diagnoses included (1) hepatic cirrhosis, postnecrotic type, (2) fibrinous pericarditis, (3) cardiomegaly, (4) non-specific calcification and fibrosis of the left auricle and the aortic valves, (5) widespread pulmonary arteriovenous fistulas with dilatation of the pulmonary vessels, (6) subacute glomerulonephritis, and (7) acute and chronic pancreatitis.

COMMENTS

Cyanosis and clubbing of the digits are phenomena known to occur in association with long standing disease of the liver. Clubbing of the fingers occurs rarely in persons with alcoholic (or portal) cirrhosis, more frequently in these with juvenile cirrhosis and primary biliary cirrhosis, and it has been reported to occur in persons with liver abscess, stricture of the bile ducts and thrombosis of the hepatic and portal veins.[2–5]

The first description of cyanosis and clubbing associated with cirrhosis in the absence of cardiopulmonary pathology was by Flückiger from Kussmaul's clinic in 1884.[6] This occurred in a thirty-seven year old woman who had cyanosis and clubbed digits for five years. Physical examination of the lungs, heart and the main vessels showed no abnormalities. Following an episode of what appeared to be hematemesis and hepatic coma, she died. Postmortem examination revealed extensive cirrhosis of the liver, believed to be due to syphilis. No disease could be demonstrated in the heart or within the parenchyma of the lungs but the pulmonary veins were grossly distended and without other change. In addition to esophageal varices and marked dilatation of the superficial abdominal veins, considerable distention of the entire venous system, including veins of the brain, spinal cord and meninges, was noted. The examiners could find no etiologic explanation for the generalized venous distention. In 1895 Gilbert and Fournier[7] reported the frequent association of cyanosis and clubbing in cases of hypertrophic biliary cirrhosis in children. The frequency of occurrence of cyanosis and clubbing in cirrhosis is not well established. Perozzi[8] collected a series of forty-seven cases of "juvenile cirrhosis" from records of the University of Minnesota Hospitals and the Mayo Clinic. She found five cases associated with cyanosis and clubbing; one case was associated with a primary cardiac disorder. In this series the rate of cyanosis and clubbing associated with cirrhosis, in the absence of primary disease of the heart or lungs, was 8.5 per cent.

The etiology of the cyanosis and clubbing in cirrhosis has remained obscure. Evans and Sheldon[5] reported such a case in a twelve year old girl in whom no disease of the heart or lungs was demonstrated. The findings in this case included (1) normal spectroscopic findings of the circulating hemoglobin, (2) normal oxygen combining power of the blood, (3) the presence of dyspnea, (4) the development of cyanosis at age eleven (at least three years after the first attack of jaundice), and (5) decreased femoral arterial oxygen saturation. They suggested that this presented the picture of a pulmonary lesion but could find no published postmortem reports which would account for it. This patient was still living at the time of the report.

Snell[9] and Snell and Starkey[10] observed that arterial unsaturation is common among patients with cirrhosis of the liver and other conditions involving severe parenchymatous hepatic damage. The degree of desaturation roughly paralleled the extent of the degenerative process in the liver. In these patients the known circulatory changes, ascites and edema does not appear to account for the unsaturation of the blood. They

suggested that either some interference in gaseous exchange in the lungs or an abnormality of the blood itself was present. At autopsy no pulmonary disease could be demonstrated. Subsequent work by Keys and Snell[11] has shown that in cirrhosis with arterial unsaturation there exists a displacement to the right in the position of the oxygen dissociation curve: the oxygen affinity of the blood is reduced. Gaensler[12] has also reported the presence of arterial unsaturation in cases of disease of the liver but others have not been able to confirm the observation.

Instances of primary pulmonary vascular disease associated with cirrhosis of the liver have not been found in a review of the literature. It is quite likely that the presence of arteriovenous shunts could be readily overlooked by ordinary methods of gross and microscopic examination. In the present case the findings in the lungs as judged by routine pathologic sections were unremarkable even though vascular shunts were specifically sought. Injection studies were essential for their demonstration. A search of the literature has failed to reveal reports of injection methods applied to the pulmonary vascular bed in cirrhosis. In the present investigation injection casts of the pulmonary vascular beds in four other cases of cirrhosis were made. These failed to reveal the presence of any vascular abnormalities comparable to the findings in the present case.

Most reported investigations of injection studies of the pulmonary and bronchial vascular systems have been limited to normal lungs or to the lungs of patients with disease of the heart or lungs.[13–20] The development of bronchial artery–pulmonary artery collateral circulation and/or the development of bronchial vein–pulmonary vein collateral drainage has been observed in chronic disorders of the lungs or heart. The development of pulmonary artery–pulmonary vein precapillary collaterals has not been known to occur except in pulmonary arteriovenous fistulas of the classical type. It is of interest that transpleural collateral circulation often develops at the site of adhesions. In such instances Liebow and associates[21] observed that the arterial collateral circulation connects directly to the pulmonary arteries; the venous collateral circulation connects directly to the pulmonary veins. Arterial collateral channels are never found connecting with the venous circulation, or the reverse; hence such adhesions do not result in a short-circuiting of the pulmonary blood flow. This observation is of fundamental importance in the consideration of the origin of the pulmonary artery–pulmonary vein channels demonstrated in the present case of cirrhosis. The question may well arise as to whether or not these collateral channels result from the opening up of pre-existing structures or whether they formed *de novo*. The latter interpretation would suggest vascular proliferation.

The existence of precapillary anastomotic channels between the pulmonary arteries and veins in normal lungs was claimed by Von Hayek[22,23] several years ago. Giampalmo[24] concluded that recent evidence supports Von Hayek's contention that such channels do exist within the parenchyma of the lung in intralobular, interlobular and subpleural locations. The observations of Tobin and Zariquiery[25] also indicate that such communications do exist. They injected glass spheres many times the diameter of the accepted capillary size into the pulmonary artery and recovered some of these spheres in the pulmonary vein. This has also been demonstrated in the lungs of certain animals.[26] The largest spheres recovered in the pulmonary vein of the human lungs measured 500 μ in diameter. By the use of a radiographic technic the locations of these shunts were determined. No anastomotic channels could be demonstrated in the hilar region nor along the larger branches of the pulmonary arteries and veins. At the apex of the lobular subdivision of the bronchopulmonary segments, anastomoses up to 500 μ were found. Smaller shunts of 50 to 100 μ were located at the level of the smaller bronchi and the respiratory bronchioles; the smallest ones, 20 to 25 μ, were near the alveolar sacs and alveoli. Anastomoses up to 200 μ were found in the subpleural region. Precapillary communications between the pulmonary arteries and veins have not been reported in the extensive studies of Liebow in which plastic injection masses were employed.

Tobin[27] has shown that in the normal human embryo and fetus there exist anastomoses between the pulmonary arteries and bronchial arteries, the pulmonary veins and bronchial veins, and probably between the pulmonary arteries and veins. He believes that these precapillary communications between the pulmonary arteries and veins may remain intact and become functional at a later date. The occurrence of the latter is suggested by the findings in his case of a thirty-six year old man

who had experienced intermittent attacks of cyanosis. Postmortem examination disclosed the existence of a short patent ductus. A striking feature was the absence of pathologic changes in the walls of the pulmonary arteries, suggesting that pulmonary hypertension had not existed. Arteriovenous shunts were demonstrated by the passage of glass beads many times the diameter of capillaries from the pulmonary artery to the pulmonary vein. These shunts were also demonstrated in a plastic cast of the lung. Such vascular channels presumably prevented pulmonary hypertension which would ordinarily be encountered in a patient with a patent ductus arteriosus.

Giampalmo[24] and Lindskog,[28] in recent reviews on pulmonary arteriovenous fistulas of the classical type, present excellent descriptions of typical pathologic findings. In this well defined entity the abnormal channels are more or less "focal" although frequently multiple, are usually subpleural in location and show degenerative changes in the vessel walls. This condition has never been reported in association with definite hepatic cirrhosis and the typical changes as described are fundamentally different from the findings in the case presented in this report.

The occurrence of abnormal pulmonary arteriovenous shunts in the present case is of interest in relation to the occurrence of other vascular abnormalities commonly associated with disease of the liver, especially spider nevi. Bean[29–34] has described the vascular spiders in pregnancy and dietary insufficiency and in apparently normal persons as well as in those with cirrhosis of the liver. When he compiled his clinical data on cases of cirrhosis according to the presence or absence of spider nevi,[31] the only significant difference in his two groups was the observation of collateral veins over the abdomen in twice as many patients with spider nevi as in those patients without them. The presence of esophageal varices appears to be similarly correlated with the presence or absence of spider nevi.[35] These findings do not appear to be related to the type of cirrhosis, but the frequency of spider nevi is increased in association with long-standing cirrhosis and splenomegaly.[31,36] The presence of distended superficial abdominal veins and esophageal varices has long been accepted as clinical evidence of portal hypertension, but Bean and other observers suspect that additional forces may be operating which favor the development of collateral vascular channels in the presence of obstruction. Numerous phenomena in patients with cirrhosis suggest an endocrinologic imbalance; this has been considered one of the possible factors concerned with the development of vascular changes. No available objective evidence supports such a supposition; hormonal influences probably play no more than a secondary role. The great majority of patients with cirrhosis and associated cyanosis and clubbing exhibit one or more of the vascular stigmas of cirrhosis and, as Gilbert and Fournier[7] first pointed out, retarded sexual development is of frequent occurrence in juvenile cirrhosis. All these changes were clinically evident to a marked degree in this patient.

Disturbances of the cardiovascular system in persons with cirrhosis would not appear to be limited to those vascular alterations just discussed. Some patients with cirrhosis who did not show evidence of co-existing disease of the heart or acute gastrointestinal bleeding exhibit an elevated resting cardiac output. Kowalski and Abelmann[37] found this to be present in one-third of their series of patients, and it was associated with high stroke volume, normal blood pressure, low peripheral vascular resistance and decreased peripheral arteriovenous oxygen difference. The average heart size and the size of each chamber was in the upper range of normal. They conclude that this is the result of generalized peripheral arteriolar dilatation, acting in effect like multiple arteriovenous fistulas in parallel, a condition similar to that seen in certain deficiency states.[38] Abelmann and his associates[39] studied the hemodynamic response of mild to moderate exercise in patients with cirrhosis. The tendency of the blood flow to be increased out of proportion to the oxygen consumption observed while the subject was at rest tended to persist or even increased during exercise. Snell and Starkey[10] had previously reported that they found high normal cardiac output values in three patients with cirrhosis and ascites that they studied. The cardiac output in the patient described in the present report was strikingly increased. All this evidence suggest generalized vascular alterations in cases of long-standing cirrhosis of the liver.

SUMMARY

Clinical and pathologic findings in a case of juvenile cirrhosis associated with cyanosis, clubbing of the digits, greatly elevated cardiac output

and a long bruit are presented. Although careful examination of the lungs at necropsy, both grossly and microscopically, failed to reveal any significant pathology, injection of a plastic solution into the pulmonary vessels revealed the presence of numerous abnormal vascular channels connecting the pulmonary arteries and veins. Thus the presence of multiple arteriovenous fistulas in the lungs, suspected and sought for during the patient's life, was finally confirmed.

Pulmonary arteriovenous fistulas of the classical type may be multiple. The findings in the present case suggest an entirely different process. A review of the literature has failed to reveal similar reported cases, probably because injection studies of the pulmonary vascular tree have not been performed in instances of juvenile cirrhosis associated with cyanosis and clubbing.

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Aggravation of Clinical Manifestations of Folic Acid Deficiency by Small Daily Doses of Vitamin B_{12}*

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THE treatment of addisonian pernicious anemia by folic acid alone will in due course and in a large proportion of patients be followed by the appearance of either a hematologic or neurologic relapse (or both) as a manifestation of an increasing deficiency of vitamin B_{12}; in some patients glossal relapse has also been described.[1–5] The clinical observations of Vilter et al.[2] indicate that the responsiveness to vitamin B_{12} of patients with pernicious anemia is diminished following prolonged folic acid therapy; the laboratory measurements of Lear[6] have demonstrated that the serum levels of vitamin B_{12} are decreased after prolonged folic acid administration. Thus several investigations suggest that the administration of folic acid may provoke more severe depletion of vitamin B_{12} by increasing the metabolic demands for the latter.

Two cases are reported herein, one of addisonian pernicious anemia and one of nutritional megaloblastic anemia, in which somewhat the reverse situation apparently obtained; severe, painful glossitis, atrophy of the lingual papillae and cheilosis developed during the administration of small daily doses of vitamin B_{12}. These lesions were promptly reversed to normal upon the administration of folic acid.

CASE REPORTS

CASE 1. A. M. (Cleveland City Hospital No. U-2149), a sixty-nine year old white widowed woman, was admitted to the hospital because of weakness. The patient was apparently well and able to care for herself until approximately six months prior to admission at which time the family observed that the patient began to "slip mentally" and intermittently began to be forgetful. She was, however, able to live alone until two months before admission when she had to be assisted back to her room because of weakness and difficulty in walking. Nevertheless, the patient continued to care for herself until approximately two weeks prior to admission when she collapsed from weakness. From then until hospitalization she was bedridden. Her family noted that she was extremely pale and ate practically nothing during this time. Inquiries made among the neighbors and subsequently confirmed by the patient revealed that she had subsisted on a completely inadequate dietary intake for more than six months. There was no history of alcoholism. The past history and family history were non-contributory.

The temperature, pulse and respirations remained normal throughout the hospital stay; the blood pressure was 130/50 mm. Hg. The patient appeared chronically ill and, although disoriented as to time and place and unable to respond consistently or coherently, she seemed to be in no acute distress. There was marked pallor of the skin, mucous membranes and nailbeds, the skin being described as pale lemon-yellow in color. The pupils were round, regular and reacted to light; the fundi could not be seen due to the patient's inability to cooperate. The tongue was very smooth and was completely denuded of filiform and fungiform papillae; it was pale without signs of inflammation. The patient was edentulous. There was moderate cheilosis. The chest was clear to percussion; a few crepitant rales were heard in the left lower base. The heart was enlarged 2 cm. to the left of the mid-clavicular line; the sounds were of good quality and the rhythm regular. A grade IV rough systolic murmur was heard loudest at the apex and transmitted over the entire precordium; no other murmurs were noted. The liver was palpable 2 cm. below the right costal margin in the mid-clavicular line; kidneys and spleen were not palpable. Rectal and pelvic examinations were normal. Neurologic examination showed physiologic active reflexes throughout; the Babinski test gave a plantar response. Tests for vibratory sensation were difficult to interpret

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although apparently it was consistently present in the arms and legs. Urinanalysis showed a specific gravity of 1.012, pH 7.4, negative for albumin, sugar, bile and acetone, and no formed elements were seen in a spun sediment. Studies of the peripheral blood showed a red cell count of 0.89×10^6/mm.^{3}; hemoglobin 4.3 gm./100 ml.; hematocrit 12.2 per cent. The erythrocyte indices were: mean corpuscular volume 137 cu. μ, mean corpuscular hemoglobin concentration 35 gm./100 ml., MCH 48 $\mu\mu$g. The icterus index was 7.5 units. The white cell count was 5.5×10^3/mm.^{3}, and the differential count showed the following percentage distribution: neutrophils 75, bands, 5, eosinophils 1, small lymphocytes 15, adult monocytes 3, young monocytes 1. The platelets appeared markedly decreased on examination of the smear. The erythrocytes showed marked variation in size and shape, definite macrocytosis with frequent oval macrocytes well filled with hemoglobin. The blood urea nitrogen was 15.8 mg./100 ml.; fasting blood sugar 88 mg./100 ml.; total protein 6.3, albumin 4.3, and globulin 2.0 gm./100 ml. The blood Kline reaction was negative. An electrocardiogram showed a ventricular rate of 80 per minute with evidence of mild left ventricular hypertrophy and strain. X-ray examination of the chest showed no evidence of pulmonary infiltration; the heart was moderately enlarged. The stools were brown and guaiac-negative. The bone marrow showed erythroid hyperplasia with abnormal erythrocyte maturation consistent with a maturation factor defect: abnormal proerythroblasts ("megaloblasts"), myelocytes and megakaryocytes were noted. Following the transfusion of 250 ml. packed red cells the erythrocyte count was 1.71×10^6/mm.^{3}, hemoglobin 6 gm./100 ml. and hematocrit 19 per cent. Gastric aspiration yielded a fasting sample of 63 ml. of clear juice containing 41 units of free hydrochloric acid. The pH of this specimen was 2.4. One hour after the injection of histamine 120 ml. of juice were obtained that titrated to 67 units of free hydrochloric acid. This specimen had a pH of 1.5. "Prophylactic" procaine penicillin was given during the entire hospital stay.

Because of the macrocytic type anemia associated with leukopenia, thrombocytopenia and the bone marrow findings described, an erythrocyte maturation deficiency was considered. Combining the demonstration of a gastric juice normal in appearance, volume and free acidity with the prolonged extremely poor food intake, the most reasonable etiologic diagnosis appeared to be nutritional megaloblastic anemia secondary to dietary lack of folic acid or of vitamin B_{12} (extrinsic factor), or both. Blood taken at this time for vitamin B_{12} determination* subsequently showed no detectable vitamin B_{12} activity. Accordingly the patient was given 1 μg. of vitamin B_{12} intramuscularly daily. On the sixth day (Fig. 1) of therapy the reticulocytes increased and a peak of 4.9 per cent was reached on the eleventh day of therapy. There were no significant changes in the other peripheral blood counts. During the course of vitamin B_{12} therapy the patient complained more and more bitterly of a painful tongue and by the twelfth day of therapy the tongue was so painful that she was unable to ingest even liquids. The lingual atrophy persisted with complete absence of papillae. (Fig. 1.) The tongue became fiery and beefy red; and fissures which cracked and bled appeared at the corners of the mouth. Because of these clinical signs and symptoms and the lack of an adequate reticulocyte response to the intramuscularly administered vitamin B_{12} the patient was given folic acid, 5 mg. orally per day. A prompt reticulocyte rise ensued, reaching 14 per cent on the sixth day of folic acid therapy. This was accompanied by a marked increase in well-being with return of appetite and steady elevation of the hematocrit, hemoglobin and red count. There was dramatic subsidence of the painful glossitis and by the fifth day of therapy the redness had disappeared so that the patient was able to ingest solid foods. Rapid regrowth of the lingual papillae occurred and at the end of the first week of folic acid therapy the tongue appeared normal. Similarly, the cheilosis disappeared, only a trace of the lesions remaining at the end of the first week of therapy. Hematologic and clinical (including mental) recovery was uneventful thereafter.

* We are indebted to Dr. Arnold Lear of the Thorndike Memorial Laboratory, Boston City Hospital, for this determination.

Case II. J. K. (Cleveland City Hospital No. U-44228), a sixty-seven year old white unmarried man, came to the hospital because of weakness, dizziness and anorexia. The patient stated that he had been in apparent good health until five months prior to admission, at which time he noted the onset of anorexia and from then on a gradual increase of generalized weakness. Two months prior to admission he was troubled by mild swelling, numbness and tingling of the lower extremities. During the month prior to admission he noted increased dizziness and, on several occasions when moderate exertion was necessary, severe precordial distress which was promptly relieved by rest. For the previous ten or twelve months he had infrequently experienced brief episodes of severe pains radiating from both flanks to the mid-epigastrium, occasionally followed by vomiting. There was no history of alcoholism. Family and past history were essentially non-contributory.

Temperature, pulse and respirations remained normal throughout the hospital stay, the blood pressure was 110/50 mm. Hg. The patient was a well developed, white-haired, blue-eyed man appearing the stated age, not acutely ill and in no apparent distress. The skin, mucous membrane and nailbeds were markedly pale and the skin was described as having a lemon-yellow tint. The pupils were round, regular and reacted to light; the fundi could not be seen because of corneal opacities. The tongue was

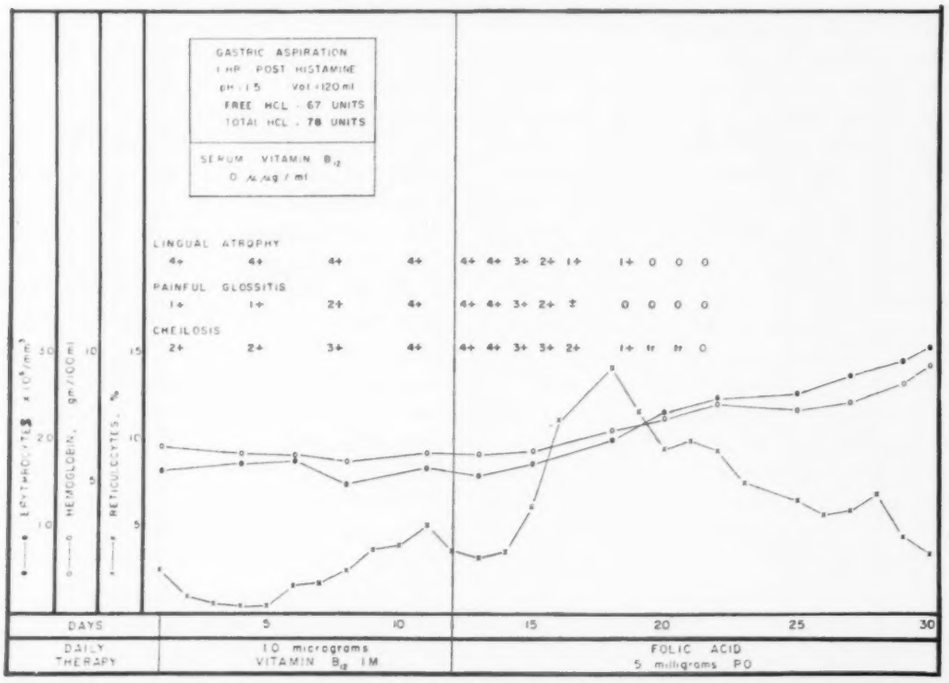

FIG. 1. Case I. Nutritional megaloblastic anemia. The deficiency manifestations of lingual papillary atrophy, painful glossitis and cheilosis are graded on an arbitrary one to four plus basis and the variations in severity of the signs and symptoms are indicated in relation to the therapy and hematologic response.

pale and smooth at the edges but papillae were present in abundance on the dorsal surface of the tongue. The patient was edentulous. There was no cheilosis. The lungs were clear to auscultation and percussion. The heart was percussed in the anterior axillary line in the fifth intercostal space; the rhythm was regular and a grade III apical systolic murmur was present and transmitted over the precordium. The liver was palpable one fingerbreadth below the right costal margin in the mid-clavicular line; the spleen and kidneys were not felt. There was a large, indirect left inguinal hernia. Rectal examination was normal. The reflexes were active and physiologic throughout both arms and legs; vibration and position sense were intact. The Babinski test elicited a plantar response. Laboratory examination showed the urine to have a specific gravity of 1.015 and to be negative for albumin, sugar, bile and acetone; the centrifuged sediment was clear except for three to six white cells per high-powered field. The stool was brown and guaiac-negative. The blood urea nitrogen was 20.3 mg./100 ml., fasting blood sugar 88 mg./100 ml., total protein 5.5, albumin 3.9, globulin 1.6 gm./100 ml. A blood Kline reaction was negative. Examination of the peripheral blood revealed a red cell count of 0.79×10^6/mm.^{3}, hemoglobin 2.9 gm./100 ml., hematocrit 9 per cent, icterus index of 13 units, reticulocytes 0.6 per cent. The erythrocyte indices were: mean corpuscular volume 114 cu. μ., mean corpuscular hemoglobin concentration 32 gm./100 ml., mean corpuscular hemoglobin 37 $\mu\mu$g. The leukocyte count was 2.4×10^3/mm.^{3}, and the differential showed the following percentage distribution: polymorphonuclears 56, lymphocytes 37, eosinophils 1 and monocytes 1. Three nucleated red cells were seen per 200 white blood cells. The platelets appeared reduced in number. The erythrocytes showed marked variation in size and shape, definite macrocytosis with frequent oval macrocytes well filled with hemoglobin. Multilobed polymorphonuclears were noted frequently. An electrocardiogram was interpreted as being within normal limits. X-ray examination of the chest showed the lung fields to be clear, with moderate cardiac hypertrophy and dilatation. Gastric aspiration showed no free acid in the fasting specimen and none one hour after histamine injection. A repeat examination made later confirmed these results. A bone marrow aspiration showed erythroid hyperplasia with abnormal erythrocyte maturation consistent with a maturation factor defect: abnormal proerythroblasts ("megaloblasts"), myelocytes and megakaryocytes were noted. Following the transfusion of one unit of whole blood and 250 ml. of packed red cells the red cell count was 1.26×10^6/mm.^{3}, hemoglobin 4.1 gm./100 ml., and hematocrit 13 per cent. "Prophylactic" gantrisin® was given from the first to the fourteenth hospital days.

Because of the typical peripheral blood and bone

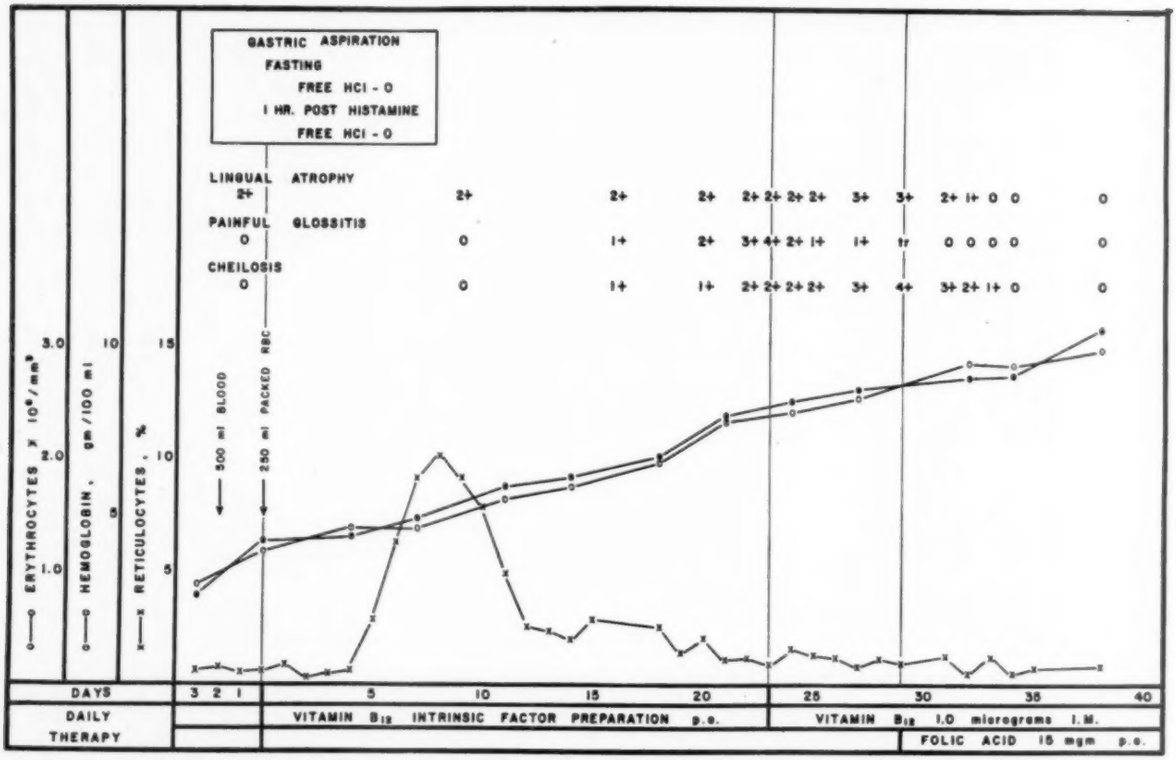

FIG. 2. Case II. Addisonian pernicious anemia. The deficiency manifestations of lingual papillary atrophy, painful glossitis and cheilosis are graded on an arbitrary one to four plus basis and the variations in severity of the signs and symptoms are indicated in relation to the therapy and hematologic response.

marrow findings and the histamine-refractory achlorhydria a presumptive diagnosis of addisonian pernicious anemia was made. The patient was given a vitamin B_{12}-intrinsic factor preparation.* On the eighth day of therapy (Fig. 2) the reticulocytes had peaked to 10.2 per cent; the hemoglobin, hematocrit and red cell count showed thereafter a steady progressive rise. On the twenty-fourth day of this therapy the red cell count had reached 2.52×10^6/mm.^{3}, hemoglobin 8.1 gm./100 ml. and hematocrit 26.2 per cent. There had been a marked improvement in general well-being and appetite concomitant with the reticulocytosis; however, there was no detectable change in the moderate lingual atrophy which had been noted upon admission. After approximately two weeks of the vitamin B_{12}-intrinsic factor preparation the patient complained of a sore tongue and it was then observed that the tongue was moderately red and that fissures had appeared at the corners of the mouth. By the twenty-third day of therapy the patient complained very bitterly of the soreness of the tongue, stating that it "burned like fire" and because of this he was unable to ingest solid foods. At this time the tongue appeared angry red and cheilosis had progressively worsened. Therapy was then changed to vitamin B_{12}, 1 μg. intramuscularly daily, for reasons to be stated.

* Supplied by Dr. Kenneth W. Thompson, Medical Director of Organon, Inc.

No further reticulocytosis occurred and the hemoglobin and red cell count continued to rise unchanged in rate. There was abrupt subsidence of the painful component of the glossitis upon institution of this therapy; however, the lingual atrophy increased in extent and degree and the cheilosis progressed to cracking and bleeding. The redness of the tongue persisted despite the absence of pain. At this point the patient was given folic acid, 5 mg. three times a day orally, and within the next five days there was marked regrowth of the papillae, the lingual manifestations had disappeared and the cheilosis had completely healed. No further reticulocytosis was noted and there was no significant alteration in the rate of rise of red count and hemoglobin during and following folic acid therapy. On the nineteenth day of therapy with the vitamin B_{12}-intrinsic factor preparation the patient had been started on a proprietary mixture of B vitamins which was administered thereafter during the remainder of the hospital stay. It consisted of thiamine 2 mg., riboflavine 3 mg., nicotinamide 20 mg. per tablet, and was given in doses of one tablet three times a day. These vitamins exerted no detectable influence on any of the patient's signs or symptoms.

DISCUSSION

Severe, painful glossitis and cheilosis appeared during the administration of small daily

doses of vitamin B_{12} to the two patients described here. In Case II (addisonian pernicious anemia) there was an associated increase in the degree of lingual atrophy, whereas in Case I (nutritional megaloblastic anemia) the lingual atrophy was complete even before therapy had been started. Although occasionally complicated by secondary infective agents, it is well documented that glossitis, atrophy of the lingual papillae and cheilosis are almost invariably the result of nutritional deficiency.[7] The fact that all of these signs and symptoms were completely and very rapidly reversed to normal upon the administration of folic acid is strong evidence that in these instances they were indeed manifestations of a folic acid deficiency. Except for vitamin B_{12}, there were no other clinical or laboratory evidences of deficiencies of other factors known to produce these signs and symptoms.[7] During the period when the patient with pernicious anemia received a combination of thiamine, riboflavine and nicotinimide, the glossitis and cheilosis increased in severity. This would rule out the possibility of a deficiency of these factors as etiologic agents since they were given in doses usually curative. Because of the times at which the antibiotics were given to both of these patients, it is unlikely that they could have played any role in the oral manifestations. During the latter part of the course of intramuscular vitamin B_{12} and the earlier part of the folic acid therapy the glossitis of the patient with nutritional megaloblastic anemia became so painful that it was impossible for her to ingest solid food and, for a period of time, liquid food, so that parenteral hydration had to be instituted.

For both patients it would then seem highly unlikely that any nutritional factors other than the vitamin B_{12} and the folic acid could have contributed to the observed changes.

It is of interest to note that although the patient with nutritional megaloblastic anemia had a gastric juice that was normal in appearance, pH, volume and free hydrochloric acid, the serum vitamin B_{12} assay by the Euglena gracilis method yielded a result indicating no detectable amount of the vitamin. A small but significant reticulocytosis followed the administration of vitamin B_{12} to this patient. Although it was not possible because of the painful glossitis to maintain this therapy long enough to demonstrate whether or not a rise in red cell count and hemoglobin would occur, the previous findings would indicate that this patient had a profound deficiency of vitamin B_{12} such as has been demonstrated in addisonian pernicious anemia[8–10] but that this deficiency had been brought about by insufficient ingestion of food extrinsic factor (vitamin B_{12}) rather than by defective absorption chiefly conditioned by lack of adequate gastric intrinsic factor. The subsequent clinical and hematologic response of this patient would further indicate that an additional folic acid deficiency was present simultaneously. Folic acid administered to this patient brought about a remarkably rapid improvement in the painful component of the glossitis, lingual atrophy and cheilosis. Hematologically a good reticulocyte response was followed by increases in hemoglobin and red count and was associated with marked subjective improvement in the patient's general well-being.

Because the patient with pernicious anemia was treated with a test fraction of intrinsic factor combined with vitamin B_{12} the possibility existed that the amount of vitamin B_{12} transported across the gut and made available to the patient was sufficient to allow for a hematologic response (Fig. 2) but insufficient to provide curative doses for the lingual atrophy, glossitis and cheilosis. The fact that the manifestations increased in severity during the hematologic response makes this possibility, of itself, seem unlikely. However, to rule out this situation more adequately, 1 μg. of vitamin B_{12} was administered intramusculary daily, an amount that uniformly gives a maximal clinical and hematologic response in patients with uncomplicated pernicious anemia. No secondary reticulocytosis was noted and there was no alteration in the rate of increase in the red cell count and hemoglobin, indicating that the vitamin B_{12}-intrinsic factor preparation had provided the patient with an amount of vitamin B_{12} at least equivalent to 1 μg. per day or that the initial hematologic response had been a maximal one.[11] Although the painful aspect of the glossitis disappeared very rapidly (perhaps supplying evidence that the vitamin B_{12} was being more adequately provided during this period of intramuscular administration), there was continued progression of the lingual atrophy and the cheilosis. After seven days of the intramuscular vitamin B_{12} therapy oral folic acid was given to the patient in a total daily dose of 15 mg. The vitamin B_{12} was continued throughout this period to obviate the complication that since the atrophy and cheilosis had become worse during

vitamin B_{12} administration they might possibly improve if the vitamin were stopped. It can be noted from the patient's chart (Fig. 2) and clinical description that with folic acid therapy the atrophy and cheilosis subsided remarkably rapidly, there being again no significant change in the rate of production of reticulocytes, red cells or hemoglobin which had, however, by then reached a level at which a further reticulocyte rise would not ordinarily be expected.

From the observations made on these two patients it would then seem most reasonable to conclude that both had a severe double nutritional deficiency of folic acid and vitamin B_{12}, and that the administration of small daily doses of vitamin B_{12} precipitated and aggravated the clinical manifestations of the folic acid deficiency.

Numerous observations have now made it clear that the administration of folic acid to patients with pernicious anemia will in a large proportion of cases be followed at some time by a hematologic, glossal and neurologic relapse as a manifestation of vitamin B_{12} deficiency.[1–5] In addition, it has been reported by Vilter and his associates[4] that the hematologic relapse with folic acid is associated with a hypocellular, non-megaloblastic bone marrow and that the response to vitamin B_{12} is a slow one unassociated with a marked reticulocyte response. These workers interpret such observations " . . . as supporting the hypothesis that folic acid must work by a mass action effect pushing the reaction [outlining the synthesis of nucleic acids from glycine, formate and the nitrogen pool] to completion, thereby further depleting vitamin B_{12} and aggravating the neurologic disorder which must be produced primarily by a vitamin B_{12} deficiency. This severe deficiency is reflected by an acellular non-megaloblastic bone marrow which responds only slowly to replacement therapy with the vitamin B_{12}." Some workers believe that folic acid alone may have a neuropathic effect in pernicious anemia. Although there is suggestive evidence for this, it cannot be stated with certainty on clinical grounds that folic acid therapy will bring on or precipitate rather than merely allow for the development of a more profound vitamin B_{12} deficiency in pernicious anemia. Nevertheless, the work of Lear[6] concerning the effect of folic acid on serum vitamin B_{12} concentrations in pernicious anemia would indicate that the administration of folic acid "may lower the serum vitamin B_{12} level possibly by accelerating the utilization of vitamin B_{12}."

The rapidity of development of the glossal manifestations in the patients herein described suggests that by inducing a hematologic response with small doses of vitamin B_{12}, the folic acid present but somewhat deficient in other areas, would preferentially be diverted to the hematopoietic system, thereby inducing a more severe deficiency elsewhere. Although not exactly comparable, the work by Heath and Taylor,[12] and James et al.[13] might be adduced in support of this hypothesis. These workers demonstrated that tissue and plasma proteins are reduced to supply material for hemoglobin production during the early phase of induced remissions in various types of anemias.

It should be pointed out that in these present studies small daily doses of vitamin B_{12} were administered—approximating 1 μg. per day. It is entirely conceivable that amounts greatly in excess of this would have induced a remission in the clinical signs and symptoms. This possibility would be entirely in keeping with the studies of Vilter et al.[2] demonstrating that when hematologic or glossal relapse occurred during folic acid therapy a temporary remission could be induced by the administration of considerably larger amounts of folic acid, presumably by forcing the utilization of the other factors required for maturation, growth and multiplication.

The clinical course of events in the patients described here indicates that administration of small doses of vitamin B_{12} precipitated the manifestations of folic acid deficiency presumably by a mass action effect similar to that which has been proposed by Vilter et al. By illustrating the circumstances in which the administration of vitamin B_{12} can augment or aggravate the manifestations of a folic acid deficiency, it is suggested that these clinical and hematologic observations furnish additional evidence concerning the reciprocal nature of the relations between vitamin B_{12} and folic acid, working through possible essential links in a metabolic chain which ends in the synthesis of nucleic acids throughout various body tissues but especially in the hematopoietic tissues.

SUMMARY

Two patients are reported, one with addisonian pernicious anemia and one with nutritional megaloblastic anemia, in both of whom severe painful glossitis and cheilosis developed

during the administration of small daily doses of vitamin B_{12}. In both patients the lesions were promptly reversed to normal upon the administration of folic acid.

It is suggested that the clinical and hematologic observations made in these patients provide additional evidence for the reciprocal relation between vitamin B_{12} and folic acid acting upon a metabolic chain that results in the production of nucleic acids throughout various tissues of the body but especially in the hematopoietic tissues. In the cases described, administration of small doses of vitamin B_{12} precipitated and aggravated the manifestations of folic acid deficiency.

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Thrombotic Thrombocytopenic Purpura with a Positive Coombs' Reaction*

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THIS report concerns a case of thrombotic thrombocytopenic purpura with a positive Coombs' reaction. The occurrence of this phenomenon has been noted in only two other instances of the fifty-five cases reported.[1] This observation should lessen the emphasis placed on a positive Coombs' test in differentiating this condition from acquired hemolytic anemia or thrombocytopenic purpura, with which it may be confused.

Reviews of the literature and general discussions of thrombotic thrombocytopenic purpura have been published by Rackow et al.,[2] Barondess[3] and Singer.[1]

CASE REPORT

A thirty-four year old white housewife was admitted to Maimonides Hospital of Brooklyn on October 20, 1952, because of sudden onset of numbness and weakness in the left upper extremity, pulling of the lower lip to the right, aphasia and faintness twenty-four hours previously. Five minutes after the onset of these symptoms she recovered without apparent residua.

The patient had been entirely well until five years before when she experienced episodes of redness, swelling and warmth of various joints. These episodes were of three to four days' duration but recurred at monthly intervals, chiefly during the premenstrual periods. She had been treated with aspirin, iodides, cortisone and various liniments. Approximately one and a half years prior to admission she had noted sensitivity of her hands to cold, associated with cyanosis of the nailbeds and swelling of the distal phalanges. In March, 1952, a physician diagnosed her condition as Raynaud's disease. During the following eight months, up to the time of admission, there was no recurrence of any joint symptoms.

For six weeks prior to admission the patient had noted unilateral occipital headaches, associated with occasional blurring of vision, a sensation that her left eye was enlarged, tinnitus, dizziness and generalized weakness. During this time she also observed petechiae and ecchymoses over the trunk and extremities. No other hemorrhagic tendency had been evident except menorrhagia at the last menstrual period.

There was a past history of mumps, appendectomy and tonsillectomy during childhood. The patient had had two normal full-term deliveries, one and five years before. Eight to ten years previously she had worked in a rubber cement factory and was exposed to carbon tetrachloride. Her mother had hypertension and her father had rheumatism. Bronchiectasis was present in one sibling, rheumatic fever in another and asthma in a third. Three other siblings and the patient's both children were alive and well.

On admission physical examination revealed a temperature of 99°F., a pulse rate of 96 and blood pressure of 110/60. The patient was a pale, white woman who had numerous petechiae and ecchymoses on the oral mucosa and the entire body area, especially the lower extremities. Several small lymph nodes were felt in the cervical region, left axilla and inguinal areas. The lungs and heart were normal. The liver and spleen were each palpable 2 cm. below the costal margin. Examinations of the rectum and pelvis showed no abnormalities. The only unusual neurologic finding was deviation of the uvula to the left.

The initial blood studies showed an erythrocyte count of 1.9 million per cu. mm.; hemoglobin 6.3 gm. per cent; hematocrit 20 per cent; leukocyte count 4,700 per cu. mm. with 53 per cent neutrophils, 4 per cent band forms, 27 per cent lymphocytes, 15 per cent monocytes, 1 per cent basophils. The platelets numbered 10,000 per cu. mm. Bleeding time was fifty minutes. Clot retraction did not occur after twenty-four hours. The peripheral blood smear showed anisocytosis, poikilocytosis, hypochromia and one normoblast per 100 leukocytes. The corrected sedimentation rate (Wintrobe) was 8 mm. per hour.

Throughout hospitalization the temperature ranged between 98.2°F. and 100.4°F. On the second day after admission the patient experienced a syncopal episode lasting three minutes. When she regained consciousness, she was aphasic. It was also noted that there was deviation of the tongue to the left, prominence of the right nasolabial fold, and weakness of the left arm. These findings disappeared in five minutes

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without residua. On the third hospital day a similar episode of focal transitory neurologic disturbance occurred. That evening the patient passed a large tarry stool. The erythrocyte count had fallen to 1.4 million per cu. mm. and the hemoglobin to 5.7 gm. per cent; 500 cc. of whole blood was administered. Cortisone therapy was begun on the following day in a dose of 75 mg. every six hours. A transfusion of 500 cc. of packed red blood cells was also given. At this time the patient had another transient occurrence of syncope, aphasia and left-sided paresis. A second episode of tarry stools occurred on the seventh hospital day.

The platelet counts varied between 10,000 to 40,000 per cu. mm. during the first week. The Rumpel-Leede test was graded 2+ or 3+; bleeding time remained prolonged; clot retraction was not present after twenty-four hours.

After four days at a level of 300 mg. daily, the cortisone dosage was continued at 50 mg. four times a day. Nine days after instituting cortisone therapy, the Rumpel-Leede test gave negative results, although bleeding time and clot retraction were still prolonged. The platelet counts remained at low levels. On the twelfth hospital day the patient again experienced marked weakness, vertigo, left-sided numbness and uncontrollable crying. Examination revealed hypesthesia and mild paralysis of the left side of the body. It was believed that cortisone had probably decreased capillary fragility but had not significantly altered the disease process. Splenectomy was therefore performed on the fourteenth hospital day. At operation the spleen was found to be twice normal in size. A small accessory spleen at the hilus was also removed. The other organs appeared normal.

Postoperatively the patient appeared comfortable. She received 1,000 cc. transfusion of whole blood. The platelet count rose to a level of 110,000 per cu. mm. The Rumpel-Leede reaction remained negative. Bleeding time diminished to one minute and thirty seconds; clotting time was two minutes and forty-five seconds.

On the second postoperative day convulsions, stertorous breathing and cyanosis suddenly developed. The patient died within five minutes.

Laboratory studies: During the first week of hospitalization the leukocyte levels varied from 4,000 per cu. mm. to 7,100 per cu. mm.; during the second week from 4,000 per cu. mm. to 14,000 per cu. mm. The differential counts were essentially unchanged. The platelets varied from 10,000 per cu. mm. to 40,000 per cu. mm. Clotting times were seven to nine minutes and bleeding times between twenty-five and fifty minutes; clot retraction did not occur. The reticulocytes were generally elevated and fluctuated between 16.0 to 26.9 per cent during most of the hospital course.

Four red cell fragility tests ranged as follows: sample of the patient's red cells showed hemolysis beginning at 0.5 per cent, 0.46 per cent, 0.58 per cent and 0.58 per cent saline solution with completion at 0.30 per cent saline solution. Three controls showed beginning hemolysis at 0.42 per cent and completion at 0.30 per cent saline solution. A direct and indirect Coombs' test performed one day after admission to the hospital gave negative results. However, the following day, before any transfusions were given, repeated direct and indirect tests were now reported as giving positive reactions. It is not known whether the first negative test was in error, since two additional subsequent tests also gave strongly positive results. The Coombs' test on the ninth day was positive in a titer of 1:256 and the indirect Coombs' 1:64. Appropriate studies showed the absence of cold agglutinins, acid and cold hemolysins, differential sheep cell agglutinins and cryoglobulins. A blood Wassermann test and agglutination tests for typhoid, parathyphoid A and B, Brucella and Proteus OX19 all gave negative results. Blood cultures taken on several occasions remained sterile. Three examinations of the blood for lupus erythematosus cells were negative.

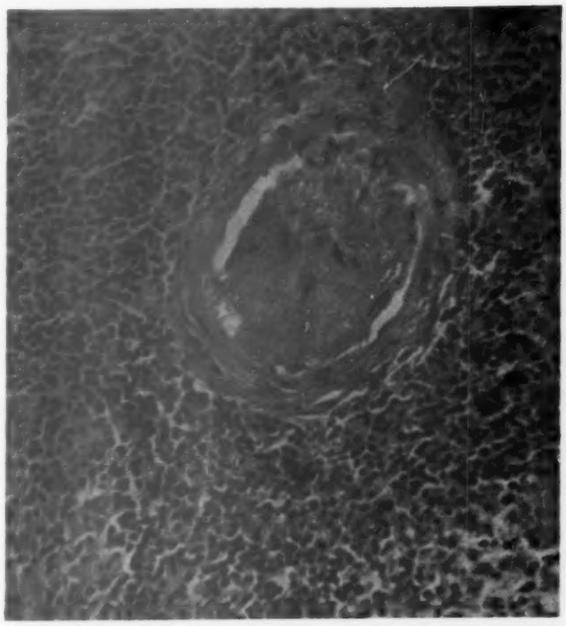

FIG. 1. Section of spleen, showing thrombotic lesion.

Bone marrow aspirations were performed on three occasions. A differential count of one of the aspirates was as follows: normoblasts 36 per cent, myelocytes 10 per cent, metamyelocytes 4 per cent, neutrophils 32 per cent, monocytes 2 per cent, eosinophils 1 per cent and lymphocytes 15 per cent. A large number of smudge cells were seen. The megakaryocytes appeared to be slightly decreased on smear. However, in another instance, when a count of nucleated cells from marrow diluted in a standard white cell pipet was performed, twenty megakaryocytes were noted in one counting chamber, an increased number by

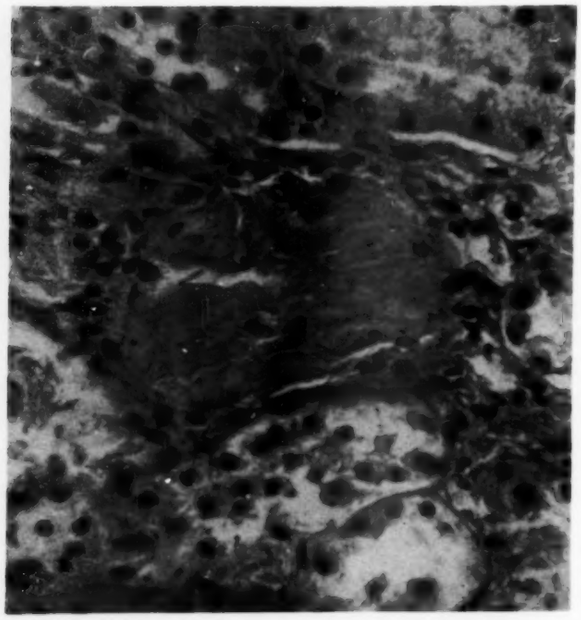

FIG. 2. Section of kidney showing thrombotic lesions.

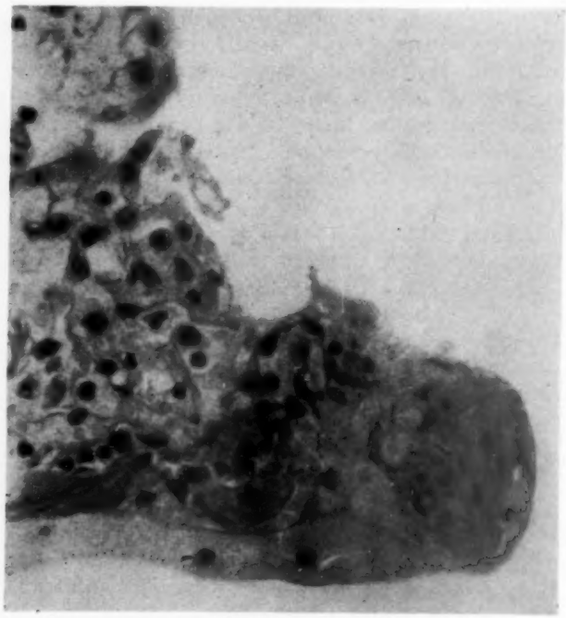

FIG. 3. Section of lung showing thrombotic lesions.

this technic. The nuclei of the stained megakarocytes appeared pycnotic and the cytoplasm granular. No vascular thrombi were reported in a formalin section of one of the marrow aspirates.

Urinalyses performed on numerous occasions were always within normal limits. Five quantitative urinary urobilinogen determinations varied from 0.5 to 0.7 Ehrlich units. Examinations of the urine for bile, hemoglobin and hemosiderin were negative. Two 72-hour fecal urobilinogen analyses indicated 300 and 500 units per 100 gm. Stool guaiac tests for blood gave positive reactions in two specimens.

The blood chemical examinations were as follows: fasting blood sugar 100 mg. per cent, blood urea nitrogen 21 mg. per cent, total protein 6.8 gm. per cent, albumin 4.1 gm. per cent, globulin 2.7 gm. per cent, prothrombin time fourteen and one-half seconds, control fourteen and one-half seconds; icteric index ranged from 10.2 to 18 units. Direct bilirubin on one occasion was 0.1 mg. per cent and indirect 0.3 mg. per cent. Cholesterol was 178 mg. per cent, cephalin flocculation 2+ to 4+, thymol turbidity 13.2 units to 15.7 units, alkaline phosphatase 6 King-Armstrong units, serum bicarbonate 28.8 mEq./L., serum chloride 106 mEq./L., serum sodium 145 mEq./L., serum potassium 3.9 mEq./L. Serial determinations of these values remained within normal levels. Examination of the serum for methemalbumin gave a positive result.

Spinal fluid examination revealed a clear fluid under normal pressure. Five red blood cells per cu. mm. were noted; total protein was 33 mg. per cent, chloride 135 mEq./L. and glucose 60 mg. per cent. Wassermann test, colloidal gold test and culture gave negative reactions.

Electrocardiogram showed non-specific T-wave changes. Roentgenograms of the abdomen, hands and chest were normal. Biopsy of skin and muscle from the right calf did not reveal any vascular thrombi or other abnormality.

Autopsy findings: No significant gross changes were noted in the abdominal and thoracic organs. Two thousand cc. of free and clotted blood were present in the peritoneal cavity and 350 cc. of blood in the pericardial sac. The brain was externally normal in appearance and showed no abnormality on cut section.

Microscopic sections of the heart, lungs, pancreas, kidneys, adrenals, bone marrow, intestine and brain revealed numerous arteriolar vessels containing thrombotic material in varying stages of organization. The vessel walls showed foci with homogeneous hyaline changes, leading to the subintimal accumulation of granular material similar to that found in the intraluminal thrombi. Sections of the spleen, kidney and lung are shown in Figures 1 to 3.

DISCUSSION

The acute illness of this patient, which encompassed a period of eight weeks, was rather typical of most other recorded cases of thrombotic thrombocytopenic purpura in its manifestations of bizarre, focal, transitory, neurologic changes, the presence of a hemolytic anemia, thrombocytopenia, purpura and gastrointestinal bleeding. The acute joint manifestations have also been reported in numerous other cases. Only one other recorded patient[4] has shown Raynaud's

syndrome. The explanation for this is not known but perhaps may be another manifestation of diffuse vascular disease. The possibility of thrombotic thrombocytopenic purpura was strongly considered in this patient during life, but neither the muscle and skin biopsy nor the bone marrow aspirate revealed vascular thrombi, as in the cases of Beigelman[5] and Cooper et al.[6] These procedures should be attempted in questionable cases.

The evidence for hemolytic anemia in this patient included an elevated icterus index, increased fecal and urinary urobilinogen, normoblastosis of the bone marrow, elevated reticulocyte count, and the presence of normoblasts and methemalbumin in the peripheral blood. The platelets were persistently depressed to levels as low as 10,000 per cu. mm. with associated features of purpura and absent clot retraction. In most of the reported cases of thrombotic thrombocytopenic purpura, megakaryocytes in the bone marrow have appeared normal in number, morphology and apparent platelet production.[1] However, the bone marrow in seven instances[3,4,6–10] revealed deficient platelet budding. In two of these,[8,10] as well as this case, the megakaryocytes appeared immature.

The administration of cortisone did not increase the platelet count but did appear to improve the vascular fragility in that the cuff test reverted from a 4+ to a negative reading. It has been suggested that increased vascular fragility as well as diminution in circulating platelets is necessary for bleeding in the presence of thrombocytopenia.

In nine previously reported cases,[5,8,10,12–15] as in this instance, the osmotic fragility of erythrocytes was increased. The variations in erythrocyte fragility observed appear to support Singer's concept of an intermittent spherocytosis in this disease. Singer[14] also demonstrated an increase in mechanical fragility of red cells in his patient.

Some observers have believed that perhaps a negative Coombs' test reaction is a feature distinguishing thrombotic thrombocytopenic purpura from acquired hemolytic anemia, in which it is usually positive. We ourselves were initially inclined to regard the Coombs' test in this light. However, although the Coombs' test gave positive results in high titer on three occasions, both by direct and indirect methods, review of the autopsy findings made the correct diagnosis unmistakable. The other two cases on record include one described by Gardner et al.[16] which was originally reported as a case of idiopathic acquired hemolytic anemia until "autopsy and restudy of the splenic sections showed the typical pattern of thrombotic purpura."[1] In the patient described by Lennox and Dacie[10] the direct antiglobulin reaction was only weakly positive.

The pathogenesis of thrombotic thrombocytopenic purpura is still a matter of speculation and even its name is controversial. Originally it was thought that the hyaline thrombi found in the arterioles and capillaries of patients with this disease were composed chiefly of platelets.[17] It was further postulated that there is normal production of platelets with overutilization, resulting in formation of platelet thrombi and thrombocytopenia. Some observers[7,12,18,19,20] are of the opinion that thrombi are the primary lesion and that the endothelial changes occurring in the vascular wall are secondary. Others[7,8,12,17] contend that the thrombi follow primary vascular changes of a degenerative nature. The strongly positive Coombs' reaction observed in our patient would also link this disorder to a large group of immunohematologic diseases.

No treatment has been curative in this condition. Although cortisone appeared to increase vascular resistance in the patient reported here, no other evidence of improvement was noted. However, the successful use of cortisone and ACTH in acquired hemolytic anemia and idiopathic thrombocytopenic purpura suggest continued trial of these agents in treating thrombotic thrombocytopenic purpura, especially when immune bodies are present.

In Meacham's case[8] splenectomy appeared to be of value in contributing to a three-year remission. The results in seven other reported cases treated by splenectomy have been discouraging.[3,12,13,18,21] In our patient, although the platelets increased postoperatively and the bleeding time returned to normal, splenectomy could not be evaluated because of her early postoperative death. Since transfusions and drugs other than cortisone and ACTH have not proved of value in treating this disease, it is our opinion that splenectomy should be given a further trial, especially if it can be done before the patient's condition has deteriorated.

SUMMARY

A case of thrombotic thrombocytopenic purpura is described in which the reaction to the

Coombs' test, both direct and indirect, was positive in high titer. This is the third such instance in a total of fifty-five reported cases.

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Successful Treatment of Gonococcic Endocarditis with Erythromycin*

Review of the Literature

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IN a review of the subject of gonococcic endocarditis in 1938 Williams[1] found that less than 150 cases had been authenticated since Thayer and Blumer[2] in 1896 first recovered the gonococcus from the blood of a patient with endocarditis. Williams at this time added an analysis of twelve cases.

During the thirteen-year period from 1936 to 1948 Jones[3] found that forty-two cases had been described in detail and thirty-nine others had been mentioned in the literature. Since 1942, with the introduction of penicillin into chemotherapy, only four detailed cases have appeared which have been treated with this and the newer antibiotics,[4–7] as well as mention of five other patients treated with penicillin.[4] This decline in reported cases reflects the decreasing incidence of the more dreaded complications of gonococcic infections since sulfanilamide was introduced. Furthermore, whereas cures of gonococcic endocarditis were a rarity prior to the sulfonamides, the prognosis is now considerably more favorable. Myers[4] in 1947 reported a cure of this disease in the first reported use of penicillin for its treatment. Subsequently penicillin and sulfadiazine were used successfully in one case reported by Dorset and his co-workers.[5] Hendlin[7] later reported a recovery following the use of aureomycin, penicillin and streptomycin.

As a further illustration of the efficacy of antibacterial agents in the management of this now fortunately rare gonorrheal complication, the first known instance of erythromycin therapy of gonococcic endocarditis is reported herein.

CASE REPORT

M. C., a twenty-three year old Negress, pregnant for the third time and in the fifth month of gestation, was admitted to the obstetric service of this hospital on August 25, 1954, with a complaint of multiple joint pains on motion. There was no history of antecedent trauma. The patient had first noted dull pain on motion of the right hip on arising five days earlier. Despite persisting discomfort in this area, she performed her housework. On arising the following day she experienced dull pain, lasting the entire day, on motion of the right shoulder, elbow, wrist and ankle, and of the left knee and proximal interphalangeal joint of the third finger. She was unable to perform her housework at this time and obtained relief only by lying motionless. Small amounts of aspirin were ineffective in relieving pain. Painful involvement of the joints mentioned continued during the following three days. The third finger of the left hand became progressively hot and swollen. Persisting joint involvement interfered with the patient's sleep and made self-feeding and ambulation difficult.

Past history revealed that pulmonary tuberculosis had been discovered following a routine school x-ray survey of the patient at the age of eleven years, necessitating a two and a half year hospitalization. Films of the chest taken at six-month intervals since her discharge with a diagnosis of arrested pulmonary tuberculosis had revealed no progression of disease. At the age of eighteen the patient experienced severe dysuria together with leukorrhea during the sixth month of her first pregnancy. At that time she received a single injection of penicillin, following which symptoms abated. The first and second pregnancies had resulted in normal delivery of a healthy child on both occasions.

On this admission to the hospital (Fig. 1) the patient did not appear acutely ill despite complaints of severe joint pain. Her temperature was 99°F., pulse rate 80 per minute, respiratory rate 20 per minute and blood pressure 100/58. There was a fusiform swelling of the proximal interphalangeal joint of the third finger of the left hand, and the finger was hot, tender and red. Tenderness, but no objective findings, was

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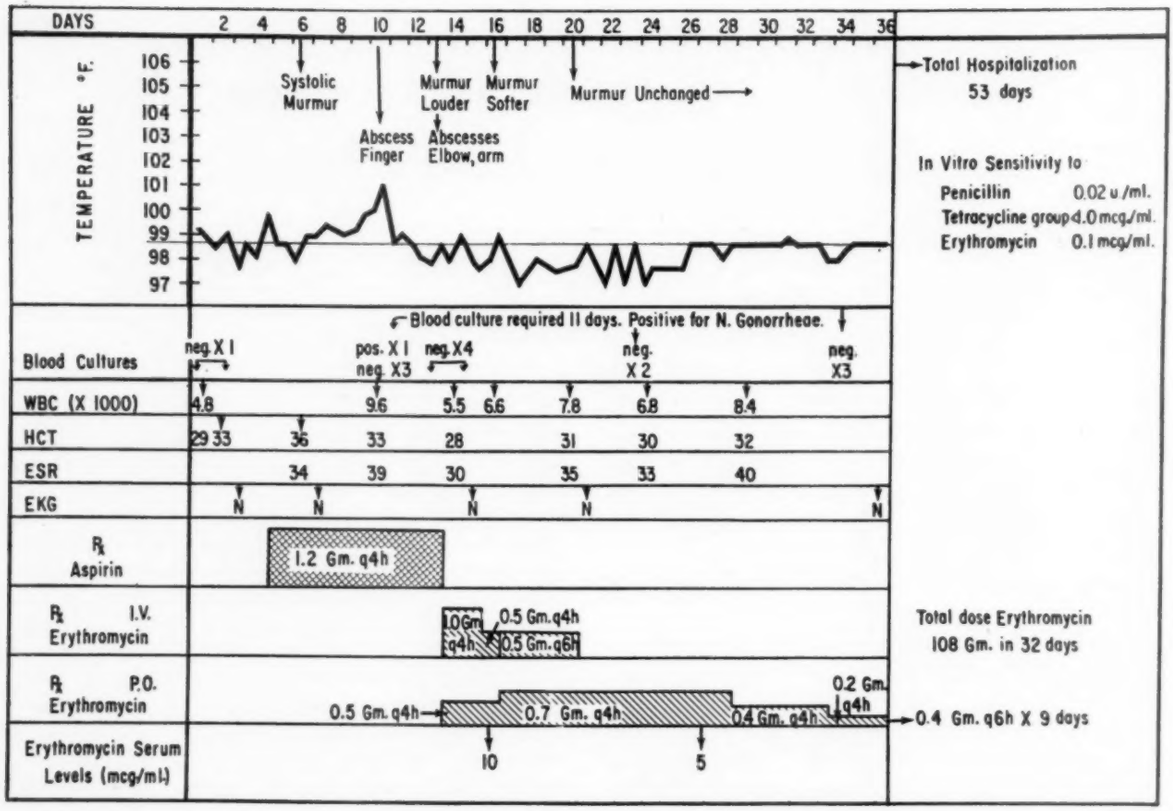

FIG. 1. Erythromycin treatment of arthritis and acute endocarditis due to Neisseria gonorrhoeae. (Patient M. C., a twenty-three year old Negro woman, five months pregnant.)

elicited at the right shoulder, right hip and left ankle. Examination of the heart and lungs revealed no abnormalities. The uterus was enlarged to a size estimated to be representative of the eighteenth week of gestation. Fetal heart tones were not heard.

Laboratory studies on admission revealed the following: hematocrit 29, white blood cell count 4,800, corrected sedimentation rate 40 mm. in one hour, urine specific gravity 1.020; qualitative tests showed no urine sugar, acetone or albumin, and microscopic examination of the urine was negative. A roentgenogram of the chest was normal. Roentgenograms of the hand showed soft tissue swelling of the third left finger at the proximal interphalangeal joint space. Serologic tests for syphilis were negative. An electrocardiogram was normal. A blood culture was negative and throat culture revealed normal flora.

The patient was placed at bed rest and demerol® was given for relief of joint pain. On the first hospital day following admission symptoms had remitted in all joints with the exception of the right shoulder and the third finger of the left hand. Careful examination by two members of the medical consulting staff revealed no abnormalities other than those previously noted. Clinically there was no cardiac enlargement. The heart sounds were normal and no murmurs were heard. No further improvement occurred over the next two days, while further examination was unrevealing. A second blood culture during this time was negative. On the evening of the fourth day the patient complained of severe malaise; her temperature was 99.8°F., pulse rate 96 per minute and respiratory rate 24 per minute. On the basis of an impression at this time of an acute rheumatic process she was transferred to the medical service and treatment was undertaken with aspirin, 1.2 gm. every four hours.

On the sixth hospital day a low, rumbling systolic murmur was detected in the third left intercostal space parasternally. The blood pressure was 110/35, right arm; 110/45, left arm. Pain in the right shoulder slowly subsided over the next several days, the murmur persisting. There was no elevation of temperature for six days following initiation of aspirin.

On the tenth day, despite continuance of aspirin, the patient's temperature spiked to 102°F. in the early afternoon without further subjective complaints or objective findings. On the eleventh day a flat 2 by 5 mm. lesion was observed on the tip of the fourth finger of the right hand, with erythematous margins and bluish center surrounded by a white border. A blood

culture was obtained hourly for a total of four. On this and the following day the rectal temperature varied from 99 to 100°F.

On the thirteenth day there was an increased intensity of the previously described murmur. The lesion on the fourth finger of the right hand had not changed in size but now had a pus-like appearance at its center; two similar lesions were found overlying the right elbow and left forearm. The material at the center of the lesion on the finger was aspirated. Polymorphonuclear leukocytes were found in great abundance microscopically but no organisms were seen. Culture of the material failed to yield growth. Four additional blood cultures were obtained.

On the fourteenth day coccic growth was detected in one of the blood cultures obtained three days earlier, although specific identification was impossible at this time. With receipt of this information antibiotic therapy was undertaken. As part of our evaluation of the clinical spectrum of erythromycin,[8] this agent was used in total dosage of 9 gm. daily, 0.5 gm. every four hours orally and 1.0 gm. every four hours intravenously, in the belief that the patient might have meningococcemia. A roentgenogram of the chest taken this day showed obscuration of the right costophrenic sinus by thickened pleura and/or fluid. The white blood cell count was 5,500. Twenty-five minutes following the first intravenous injection of erythromycin, a 1.0 gm. dose, the patient experienced a shaking chill. Her temperature was 100.4°F. A second chill occurred the following day, although rectal temperatures were normal. The patient offered no complaints during this period.

On the fifteenth day, the second day of treatment, the systolic murmur previously heard parasternally in the third left intercostal space was noted to have increased in intensity. The first heart sound at the apex was accentuated, and a third heart sound was audible in the same area. Moderate exercise produced a transient diastolic murmur at the apex with the patient in the left lateral decubitus position. An electrocardiogram was normal.

On the sixteenth day, the third day of therapy, the systolic murmur remained unchanged in intensity but was now also audible in the aortic area. Two pustular lesions were discovered overlying the right knee; other pustules appeared on the forehead. A third severe shaking chill, lasting twenty minutes, occurred on the nineteenth day, at which time the rectal temperature was 98.6°F.

On the twentieth day, the seventh day of therapy, the systolic murmur was unchanged. No new lesions were noted on the skin. The white blood cell count was 7,800, hematocrit 31 and corrected sedimentation rate 35. The rectal temperatures for the past week had remained 98° to 100°F. Symptomatic improvement had been manifested in all the joints involved, and all cutaneous lesions were receding.

On the following day the organisms previously found to be growing in a single blood culture were identified as members of the genus neisseria. Intravenous erythromycin was discontinued and oral erythromycin was increased to a dosage of 0.7 gm. every four hours.

On the twenty-third day, the tenth day of therapy, the patient was asymptomatic. Virtually complete function had returned to the right shoulder but the murmurs were unchanged. On the twenty-fifth day the systolic murmur was no longer audible over either the apex or base of the heart but still could be heard parasternally in the third left intercostal space. On the twenty-seventh day final bacteriologic identification was made of Neisseria gonorrhoeae grown in a single blood culture obtained on the eleventh hospital day. *In vitro* antibiotic sensitivity tests revealed the following:

Sensitive to penicillin in concentration of	0.02	μ./ml.
Sensitive to tetracycline in concentration of less than	1.0	μg./ml.
Sensitive to oxytetracycline in concentration of less than	1.0	μg./ml.
Sensitive to chlortetracycline in concentration of less than	1.0	μg./ml.
Sensitive to erythromycin in concentration of	0.1	μg./ml.

On the thirty-first day, the eighteenth day of treatment, grade 2, harsh systolic murmurs were present at the apex and pulmonic area. The systolic murmur was now less intense in the third intercostal space. By the thirty-fifth day the systolic murmur had resumed its harsh character and had returned to grade 3 intensity parasternally in the third left intercostal space. The apical systolic murmur was now faint. No diastolic murmur could be heard. Serial electrocardiograms continued to be normal.

Erythromycin was discontinued on the forty-fifth day, after thirty-two days of this medication. The following values were obtained for blood serum concentrations of this agent:

3d day of therapy,	10.0	μg./ml.
14th day of therapy,	5.0	μg./ml.
24th day of therapy,	0.624	μg./ml.

During the remainder of her hospitalization the patient continued to be afebrile and asymptomatic. A harsh systolic murmur of grade 2 to grade 3 intensity persisted in the third left intercostal space. After eight days of observation in the hospital following cessation of erythromycin therapy she was discharged to her home with provision for clinical follow-up.

Comments. Presented herein is the case of a twenty-three year old Negress in whom polyarticular arthritis developed during pregnancy five years after a probable primary gonorrheal

infection. Under observation a systolic murmur first appeared and later became changing in character. A diastolic murmur also made a transient appearance. Coincident with the onset of murmurs, skin lesions of probable embolic nature appeared on the extremities. One of ten cultures obtained during the initial period of observation yielded growth of N. gonorrhoeae, thereby confirming a diagnosis of gonococcic endocarditis.

Specific therapy consisted of a total of 108 gm. of erythromycin administered orally and intravenously over a thirty-two-day period. No growth was observed in nine blood cultures obtained during this course of treatment, and single blood cultures obtained on the fourth, sixth and seventh days after cessation of therapy were negative. Marked objective and symptomatic improvement occurred during hospitalization lasting fifty-three days. Two and a half months later the patient was delivered of a healthy child without further untoward event.

When last seen ten months after the onset of her illness the patient continued to be in excellent health. She had had no recurrence of arthritic manifestations and there had been no febrile episodes. Clinically, there was no demonstrable cardiac enlargement. Heart sounds were of good quality and intensity with a regular sinus rhythm of 70 beats per minute. A soft systolic murmur remained audible in the third left intercostal space. Her blood pressure was 110 systolic, 65 diastolic.

In vitro studies[8–10] demonstrating the susceptibility of the gonococcus to erythromycin have been clinically supported by reports of use of this agent in the treatment of gonorrhea.[10] This report would seem to indicate the further applicability of erythromycin in treatment of gonorrheal complications.

CLINICAL FINDINGS

Incidence. Table 1 represents a summary of the major reported series of bacterial endocarditis in which attempt has been made to establish the bacteriologic diagnosis.

Thayer[11] in 1922 reviewed 176 cases of acute and subacute bacterial endocarditis in which bacteriologic diagnoses were made and necropsies performed. He found a gonococcic etiology in 11 per cent of this series. Streptococci (all strains) were present in 57 per cent. In the remaining cases the pneumococcus was present in 14 per cent, Staphylococcus aureus in 13 per cent, Hemophilus influenzae in 4 per cent and Staphylococcus albus in 1 per cent.

In 1926 Cabot[12] stated that he had failed to find a single instance of gonococcic endocarditis in a study of 1906 autopsies performed some years earlier on patients with heart disease. One hundred eighty patients in this group demonstrated some form of endocarditis.

TABLE 1
INCIDENCE OF GONOCOCCIC ENDOCARDITIS

Author	Total Cases of Endocarditis	Cases of Gonococcic Endocarditis	Percentage
Thayer[11]	176	20	11
Cabot[12]	180	0	0
Johnston and Johnston.[13]	18	2	11
Williams[1]	38	10	26
Totals	412	32	8

The two cases of gonococcic endocarditis reported by Johnston and Johnston[13] reflect the results of 1,192 autopsies performed between 1910 and 1926, among which were eighteen cases of acute endocarditis.

Williams' report[1] in 1938 was based on 1,719 autopsies performed during the preceding twelve-year period. He found thirty-eight instances of acute and subacute endocarditis. The gonococcus was responsible in ten cases, Streptococcus hemolyticus in eight cases, Streptococcus viridans in seven cases, staphylococci in four cases, pneumococci in two cases, H. influenzae in two cases and other organisms in five cases.

Jones,[3] reviewing the world literature for the thirteen-year period from 1936 to 1948, listed the causative organisms in 212 cases of bacterial endocarditis of non-streptococcic etiology; forty-two of these were gonococcic.

Age, Sex and Race. Stone,[14] in an exhaustive review of gonococcic endocarditis, found an age range between two years and fifty-one years among eighty-five patients, with an average age of 26.4 years. Williams' twelve patients ranged from nineteen to sixty-nine years, with an average of thirty-six years. Approximately 65 per cent of all the patients reported have been men. Only two of Williams' twelve patients were

Negroes, whereas Thayer found both races equally represented in his series.

Time Relationship of Endocarditis to Primary Infection. In most instances it has been impossible to ascertain accurate data regarding this matter. Many patients either denied or were unaware of preceding venereal disease. In others the symptoms of such infections had subsided an indeterminate number of years previously. In the five instances in which Williams was able to establish a time relationship this varied from three weeks to fourteen years.[1]

Related Clinical Findings. Classically, prior to use of current chemotherapy, reports of this disease were characterized by a "septic course," with chills, sweats and remittent or intermittent fever. Daily fluctuations in temperature of as much as 8° to 10°F. commonly occurred. Horder and Gow[15] noted the frequent daily occurrence of "double peaks" in temperature which they believed to be suggestive of gonococcic septicemia. Chills were commonly present.

Coexistent arthritis was present in the majority of patients reviewed by Thayer and Williams. Arthritic manifestations usually preceded those of endocarditis, although in some cases their development occurred concomitantly. Multiple arthralgia was experienced most commonly. This sometimes subsided without sequelae or occasionally progressed in one or two joints to present characteristics of acute suppurative arthritis and associated tenosynovitis. The knees, ankles and wrists were the most commonly affected joints.

In approximately one-third of the cases reported petechiae were exhibited during the course of the illness. These lesions varied in size from minute spots to areas 1 cm. in diameter, the latter occasionally exhibiting central areas of necrosis. Other embolic phenomena were noted less frequently clinically. The most common sites of lodgment were the skin, conjunctivas, kidneys, spleen, lungs, brain and myocardium.

The presence and nature of murmurs and other classic cardiac and peripheral signs were dependent upon the location and extent of valvular involvement. Slight to moderate cardiac enlargement was frequently present. The appearance of a diastolic murmur or an unmistakable change in quality and timing of systolic murmurs during observation of the patient were and continue to be among the accepted criteria for establishment of the clinical diagnosis. In two instances in Williams' series correct antemortem diagnoses of ruptured aortic cusps were made in patients with progressive valvular destruction.

Acute nephritis was one of the most common complications of gonococcic endocarditis. Seven of Williams' twelve patients had this complication, and in five of these uremia was the main cause of death. Thayer and Stone also found this complication in most of the cases they reviewed. It was usually embolic glomerular or intracapillary in type and its development was first indicated by presence in the urine of a moderate amount of albumin, red and white blood cells, and casts.

Laboratory Findings. Leukocytosis was usually outstanding. In Williams' series the white blood cell counts ranged from 10,500 to 53,700, with an average of 21,120. Thayer found white blood cell counts greater than 20,000 in fourteen of his twenty-two patients. Stone found the highest count to be greater than 26,000 in eighteen of his thirty-seven patients. The hemoglobin value steadily declined with progression of the untreated disease. Electrocardiographic changes most commonly encountered by Williams consisted of T wave depression, and slurring and notching of the QRS complex. Albuminuria, hematuria, pyuria and casts signaled the onset of dreaded renal complications.

Duration of Illness. Thayer observed that the chronicity of gonococcic endocarditis occupied a mid-position between the acute fulminant endocarditis associated with the hemolytic streptococcus, pneumococcus and staphylococcus, and the subacute process which is usually associated with S. viridans and the influenza bacillus. Among the twelve patients in Williams' series the duration of illness prior to death was estimated as varying from ten days to fourteen weeks, with an average of five and a half weeks.

ANATOMIC FINDINGS

Varying degrees of erosion and ulceration of the valvular leaflets was the chief pathologic finding.[1] Vegetations were usually large and friable, grayish yellow, and composed of fibrin, leukocytes and gonococci. In the combined data of Kirkland,[16] Thayer[11] and Williams,[1] based on 103 reports of gonococcic endocarditis, the valvular afflictions encountered were as follows: forty-five aortic lesions, twenty-five mitral lesions, seven pulmonic lesions, one tricuspid lesion, twenty-five lesions affecting more than one valve (thirteen of these had the com-

bination of aortic and mitral involvement). Thus 90 per cent of all *single* valvular lesions involved the left side of the heart; in all cases, including those with *multi-valvular* involvement, the left side of the heart was exclusively affected 80 per cent of the time.

Thayer and Williams found slight dilatation of the cardiac chambers in a few instances, but little if any cardiac hypertrophy was noted. Thayer encountered pericarditis in four instances; Stone noted this finding in thirteen cases. This lesion was usually of the purulent variety.

Williams found areas of focal necrosis in the myocardium in six of ten postmortem examinations. In addition, Williams found two instances of ruptured cusps, two cases of embolic pneumonia and two cases of interstitial pancreatitis. Six of his patients had splenic infarcts. He found the liver to be enlarged and congested in all ten cases. Eight patients had glomerular nephritis (three intracapillary, three embolic, one acute, one acute focal); two had renal infarcts.

Among the twelve cases in Williams' study, the chief cause of death was ascribed to acute heart failure in six, to uremia in five and to cerebral embolism in one.

DIAGNOSIS

The successful diagnosis of bacterial endocarditis, regardless of etiology, is dependent upon and directly correlated with the index of suspicion of the physicians in attendance. The chief obstacle in substantiation of the diagnosis of gonococcic endocarditis is the recovery of the organism on blood culture. Even in the presence of an adequate nutritional environment, many days are often required for cultivation, following which fermentative and agglutinative reactions are required for differentiation. The problem of cultivation is further magnified when the size of the inoculum, in terms of the number of organisms in a blood sample, may be small. Yet an added impediment may be ascribed to the right-sided cardiac lesion with the capillary bed of the lungs serving as a filter before the organisms reach the general circulation.

Gonococci were grown in antemortem blood cultures one or more times in nine of the twelve cases composing Williams' series. (Five of these nine had gonococci in smears made from vegetations at necropsy.) The medium employed for blood culture consisted of dextrose-agar and yeast-broth to which ascitic fluid was added. In our laboratory, in the case reported herein, brain-heart infusion (difco®) was used, and the cultures were incubated under increased carbon dioxide tension.

Gonococcemia without endocarditis and nongonococcic bacterial endocarditis pose the major problems in differentiation of this condition. In addition, other occasional causes of temporary confusion are acute rheumatic fever, meningococcemia, miliary tuberculosis, typhoid fever, malaria and pylephlebitis.

PROGNOSIS

Freund et al.[17] estimated a mortality rate of 93.5 per cent in 1938, prior to the sulfonamide era, based on seven recoveries in 108 patients "definitely proved to have the condition." However, Futcher and Scott[18] found only three cases of authenticated recovery based on the diagnostic criteria of (1) positive blood culture and (2) diastolic murmur at the base of the heart appearing or disappearing under treatment. In the eighteen cases listed in Table II, including our own, in which an antibiotic and/or sulfonamide was employed, the mortality was approximately 40 per cent.

TREATMENT

Prior to specific therapy with antibiotics many forms of treatment were advocated and utilized in varying combinations. These included blood transfusions, gonococcic vaccines and serums, intravenous silver, mercurochrome, arsenic and artificial induction of hyperthermia. In three well authenticated cases in which recoveries were reported,[19–21] all three patients received one or more blood transfusions and two received vaccines. Table II summarizes the results of specific chemotherapy.

Of eight patients who received a sulfonamide drug prior to the antibiotic era, four survived.[18,22–25]

Myers report[4] is the only detailed instance of the use of penicillin as the sole antibiotic in therapy of gonococcic endocarditis. In addition to the recovery of his own patient, Myers wrote: "The National Research Council received reports of penicillin therapy in five cases of acute gonococcic endocarditis. Recoveries or clinical arrests were reported in three of these cases."

As also noted in Table II, there are three case reports of combinations of antibacterial agents

TABLE II

RESULTS OF TREATMENT OF GONOCOCCIC ENDOCARDITIS WITH SULFONAMIDES AND ANTIBIOTICS

Year	Authors	Agents	Dose	No. of Cases	Deaths	Recoveries
1938	Calderon-Hernandez[22]	Sulfanilamide	34.5 gm.	1	..	1
1939	Futcher and Scott[18]	Sulfanilamide		4	3	1
1939	Orgain and Poston[23]	Sulfapyridine	Approx. 200 gm.	1	..	1
1940	Beckley and McCrea[24]	Sulfanilamide	?	1	1	..
1940	Dohmen[25]	Diseptal B (Neo-uliron)	12 gm.	1	..	1
1947	Myers[4]	Penicillin	10.99 mil. u.	1	..	1
1947	National Research Council[4]	Penicillin	?	5	2	3
1949	Dorset et al.[5]	Penicillin, sulfadiazine	23 mil. u., 62 gm.	1	..	1
1953	Pereira[6]	Penicillin, streptomycin, aureomycin, terramycin®	?	1	1	..
1953	Hendlin[7]	Sulfadiazine Penicillin Aureomycin Streptomycin	Approx. 20 gm. Approx. 3 m.l. u. Approx. 96 gm. 3 gm.	1	..	1
1954	Davis and Romansky	Erythromycin	108 gm.	1	..	1
			Totals	18	7	11

used in treating this disease, with two instances of recovery[5,7] and one death.[6]

SUMMARY

1. Successful therapy with erythromycin of a patient with gonococcic endocarditis is reported.

2. Erythromycin, 108 gm., was administered orally and intravenously over a thirty-two-day period.

3. A review of the literature of gonococcic endocarditis is presented.

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Superior Vena Cava Draining into Left Atrium*

Another Cause for Left Ventricular Hypertrophy with Cyanotic Congenital Heart Disease

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DEVELOPMENTAL anomalies of the systemic return to the heart have recently been reviewed.[1] A persistent left superior vena cava commonly drains into the right atrium via the coronary sinus. It is unusual to find the superior vena cava entering the left atrium. Winter reported twenty-one cases from the world literature in which the left superior vena cava drained into a left atrium, common atrium, or sinus venosus and all showed associated septal defects. In his own series four patients in whom the superior vena cava entered the left atrium all had other complicating defects.[2] Friedlich, Bing and Blount in 1949 described four patients with complicated defects in whom cardiac catheterization demonstrated that the superior vena cava drained into the left atrium,[3] but to our knowledge no instances have been reported in which this anomaly was present without other congenital cardiac defects. A case in which postmortem examination revealed that the inferior vena cava entered the left atrium has recently been described.[4]

Clinical, hemodynamic and anatomic descriptions are presented herewith of a patient diagnosed by cardiac catheterization in whom the superior vena cava entered directly into the left atrium. This anomaly was the only congenital abnormality present.

CASE REPORT

The patient was a fifteen year old white boy who was admitted on February 26, 1955, to the University Hospitals complaining of fatigue and unexplained fever. The patient's mother had had no major illnesses during her pregnancy and the birth was uncomplicated. She had noted cyanosis of the boy's finger tips when he was still very young. Although he was the largest child and could outdo his siblings in a simple test of strength, his endurance was limited. He had excelled scholastically but six months before admission to the hospital a perceptible decline in his academic standing had occurred. The patient stated that in September 1954, he had experienced transient and intermittent afternoon fever (99° to 100°F.). He had noted easy fatigability, and shortness of breath and precordial pain with exertion. In October 1954, his local physician found that polycythemia was present. In November he entered the local hospital for cardiac evaluation. He was discharged in six days without a definitive diagnosis having been made.

Physical examination at this hospital revealed a well developed and well nourished white boy who appeared somewhat dull. The pulse was 88 per minute, the respiratory rate 20 per minute, and the blood pressure 120/70. The temperature was 100°F. but no significant temperature elevation persisted during the hospital stay. His height was 5 feet 10½ inches and he weighed 156 pounds. The patient appeared to be moderately plethoric with cyanosis of the acral parts. Down-curving of the finger nails without actual clubbing was noted. The conjunctival and retinal vessels were engorged. No cardiac murmurs were heard. The left border of cardiac dullness was at the midclavicular line. The liver was palpable 3 cm. below the costal margin and the spleen was just palpable. Laboratory evaluation revealed a hemoglobin of 19.4 gm. per 100 cc., erythrocyte count 6.61 million, leukocyte count 7.0 thousand and the differential count was

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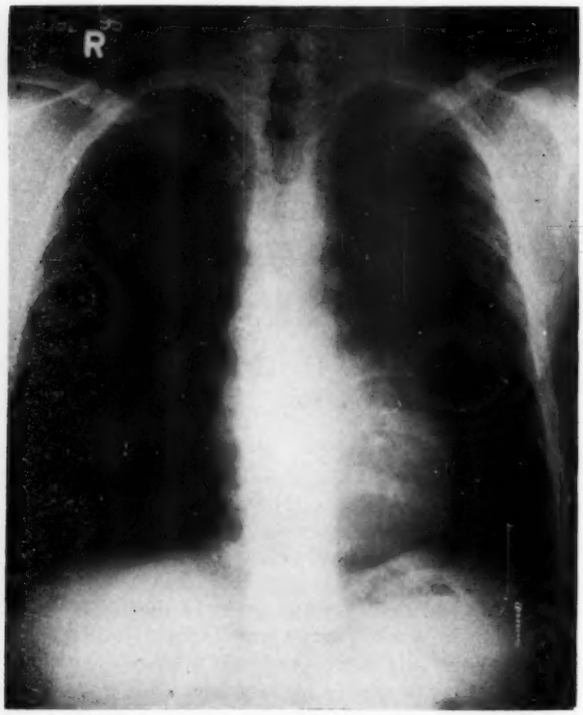

FIG. 1. Roentgenogram of the chest.

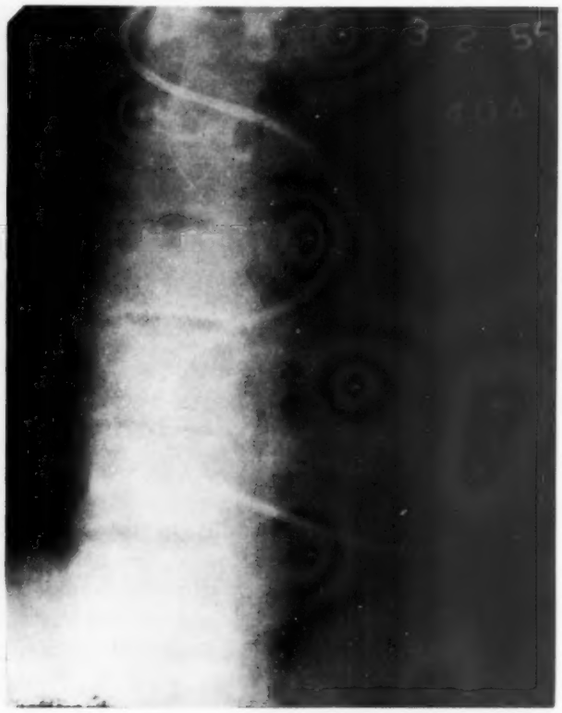

FIG. 2. Radio-opaque catheter entering the left atrium from the superior vena cava; the tip of the catheter is in the left ventricle.

TABLE 1
DATA OBTAINED DURING CARDIAC CATHETERIZATION

Catheter Site	Oxygen Saturation* (%)	Pressure (mm. Hg)	
		S.D.	Mean
Superior vena cava	78	.....	14.7
Inferior vena cava	77	.....	....
Right atrium	75	.....	4.7
Right ventricle	70	30/0	....
Pulmonary vein	100+	.....	6.2
Left atrium	89	.....	4.8
Left ventricle	92	118/−10	....
Femoral artery	90	96/72	76

Note: surface area (M^2) = 1.89; O_2 consumption (cc./min.) = 339; O_2 capacity = 23.9 vol. per cent.

Blood flow (L./min./M^2): systemic = 8.5; pulmonary = 5.3.

Shunt (L./min./M^2): R → L = 3.2 (38 per cent); L → R = 0; over-all = 3.2.

* Oxygen saturations were determined with a curvette oximeter.[5]

normal. Hematocrit was 58 per cent. A diagnosis of polycythemia rubra vera was entertained.

A teleroentgenogram revealed cardiomegaly (Fig. 1) and fluoroscopy confirmed moderate left ventricular enlargement. The electrocardiogram revealed notching of QRS complexes in standard leads II and III, T wave inversion in II, III, AVF and V_6, and a delay in the intrinsicoid deflection in the left precordial leads suggesting left ventricular hypertrophy.

Because no satisfactory explanation for the cyanosis and left ventricular hypertrophy had been found, cardiac catheterization was performed on March 2, 1955. A catheter, introduced through the left median antecubital vein, passed through the superior vena cava and approached the heart shadow in a normal manner. However, instead of descending into the right atrium it veered to the left and entered the left atrium and left ventricle. (Fig. 2, Table 1.) From the left atrium the catheter entered all four pulmonary veins but could not be made to enter the right atrium. Therefore, the catheter was introduced through the left femoral vein and the inferior vena cava into the heart. The catheter entered the right atrium in a normal way but could not be passed out of the atrium into the superior vena cava. During attempts to find the superior caval entrance, auricular fibrillation with a rapid ventricular rate began. The catheter tip was successfully placed within the right ventricle but runs of ventricular tachycardia occurred when an attempt was made to pass it into the pulmonary artery. The catheterization was terminated and the patient was given 0.8 mg. of lanatoside C (cedilanid®)

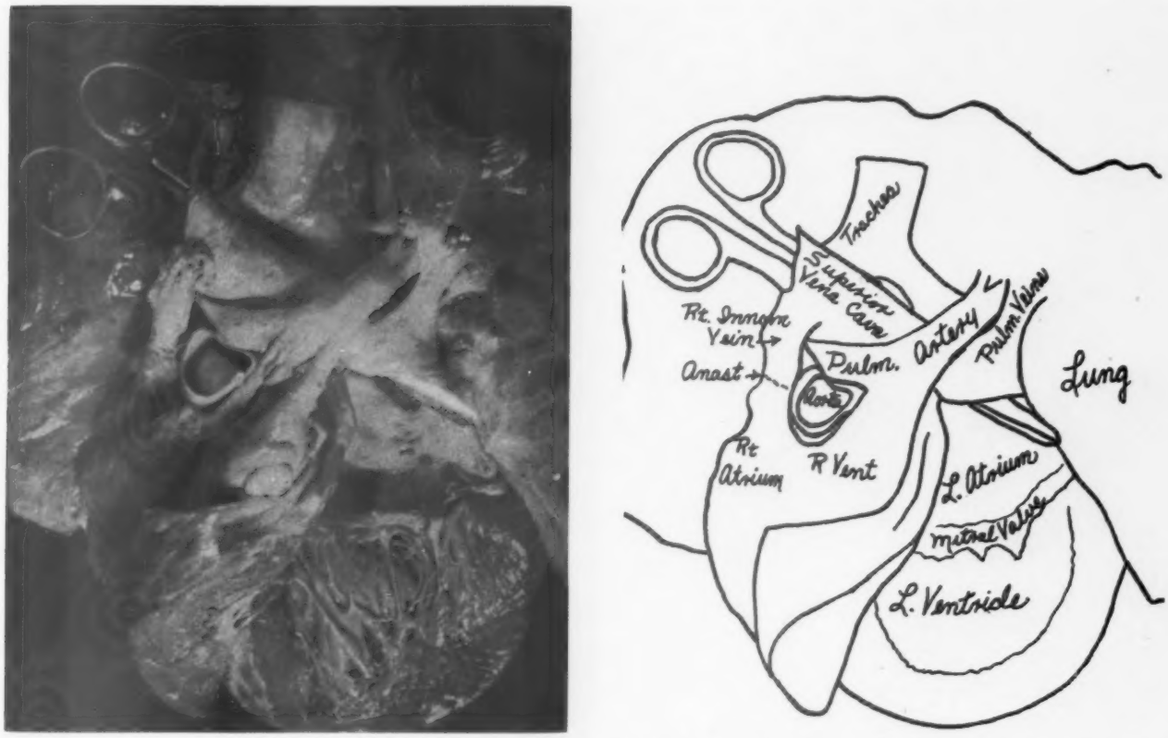

FIG. 3. Opened left atrium and left ventricle with scissors placed in the superior vena cava. Note the thickened wall of the left ventricle and the surgical anastomosis between the innominate vein and the right atrium.

intravenously. Twelve hours later normal sinus rhythm was present. It was the impression clinically at this time that the patient had a single vascular anomaly in which the superior vena cava entered directly into the left atrium, and surgery was recommended.

Surgical exploration confirmed that the superior vena cava entered the left atrium. The aorta and left ventricle were enlarged. The right atrial appendage was hypertrophied and dilated. Within the pericardium and extending upward from the cephalic end of the right atrium was a fibrous remnant of the normal superior vena cava. The surgical procedure will be described by the thoracic surgery department elsewhere. The patient died twenty hours after operation.

Postmortem examination showed that the superior vena cava entered the left atrium, (Fig. 3) and thorough examination failed to reveal any other congenital cardiac anomalies. In the right atrium opposite the fibrous remnant of the normal superior vena cava was a dimple in the endocardium, 2 mm. in diameter. (Fig. 4.) The left ventricle was hypertrophied, measuring 2 cm. in thickness.

COMMENTS

Detection of variations in the venous return has become more frequent with the use of cardiac catheterization and angiocardiography for the diagnosis of congenital disease of the heart. The embryologic development of the venae cavae has recently been reviewed by Miller, Inmon and Pollock.[1] For the most part, the persistent left superior vena cava drains either into the coronary sinus and then into the right atrium where it does not alter hemodynamics, or into the left atrium where it is associated with other abnormalities which markedly affect the pattern of circulation. In this patient the superior vena cava entered directly into the left atrium and was the only developmental defect. The hemodynamic result was a significant right to left shunt and no evidence of strain on the right heart was present; rather, the left ventricle was hypertrophied.

This case is thought to be of interest for several reasons: (1) another type of cyanotic cardiovascular anomaly producing left ventricular hypertrophy is described, and (2) the quantity of shunt as calculated here confirms the existing impression that approximately one-third of the venous return is via the superior vena cava and two-thirds via the inferior vena cava.[6] The diagnosis should be easily available to all who

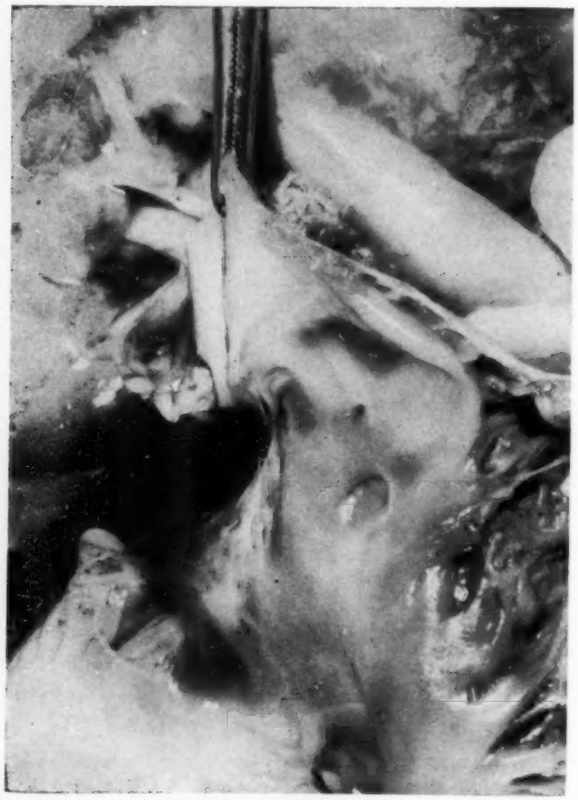

FIG. 4. The opened right atrium showing the fossa ovalis and a small dimple in the endocardium at the usual site of entry of the superior vena cava.

have access to cardiac catheterization and angiocardiography, and correction by surgery is feasible.

In this case angiocardiography was not performed because of the fear of presenting a high concentration of contrast material directly to the coronary arteries and systemic circulation,[7] and because a satisfactory diagnosis had been made by cardiac catheterization.

SUMMARY

A case of cyanotic congenital heart disease with left ventricular hypertrophy is described. Cardiac catheterization revealed a superior vena cava which drained into the left atrium. No other cardiovascular abnormality was present. The diagnosis was confirmed by operation and postmortem examination.

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Notes on the Diagnosis and Management of "Dizziness"

III. Ménière's Syndrome

1. Paroxysmal Whirling Vertigo. *This consists of sudden attacks of dizziness, often when the patient is at rest or asleep. The patient may feel that he himself is whirling or that fixed objects about him are whirling. The attack usually lasts for a few minutes; occasionally it is severe for weeks or subacute for months.*

2. Subtotal Hearing Loss. *Deafness will usually affect the high tones and it may be unilateral or bilateral. Sometimes the hearing loss is severe and also progressive.*

3. Tinnitus. *This is usually unilateral and present in the ear with greater hearing loss and is without a definite pattern.*

Fewer diagnostic errors[1] will result if a "triad of symptoms" is required of patients with suspected Ménière's syndrome. These are the symptoms of typical Ménière's syndrome:

1. Severe paroxysmal vertigo which may be of two types; either the patient feels that he is whirling or that objects about him are whirling.
2. Fluctuating subtotal hearing loss, usually affecting the higher tones, is noted at the same time as vertigo.
3. Tinnitus, usually unilateral, is associated with the deafness and dizziness.

With Ménière's syndrome there is no definite localization[2] by the Bárány (vestibular reaction) test and results of the caloric test are not diagnostic. Physical examination should rule out disease of the central nervous or cardiovascular systems before a diagnosis is made.

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1. DeWeese, D. D.: Symposium: Medical Management of Dizziness. The Importance of Accurate Diagnosis, Tr. Am. Acad. Ophth. *58*:694 (Sept.-Oct.) 1954.

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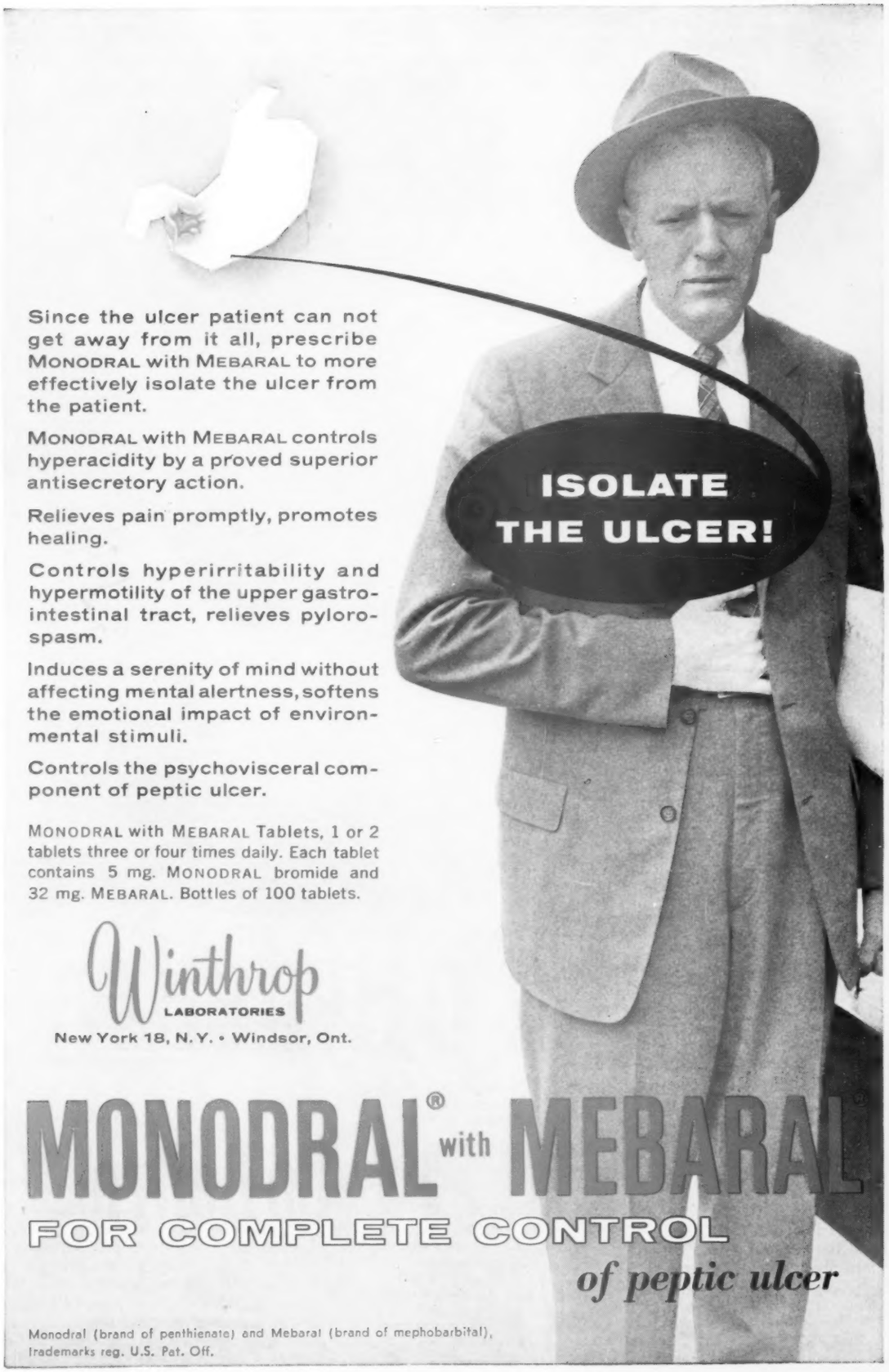

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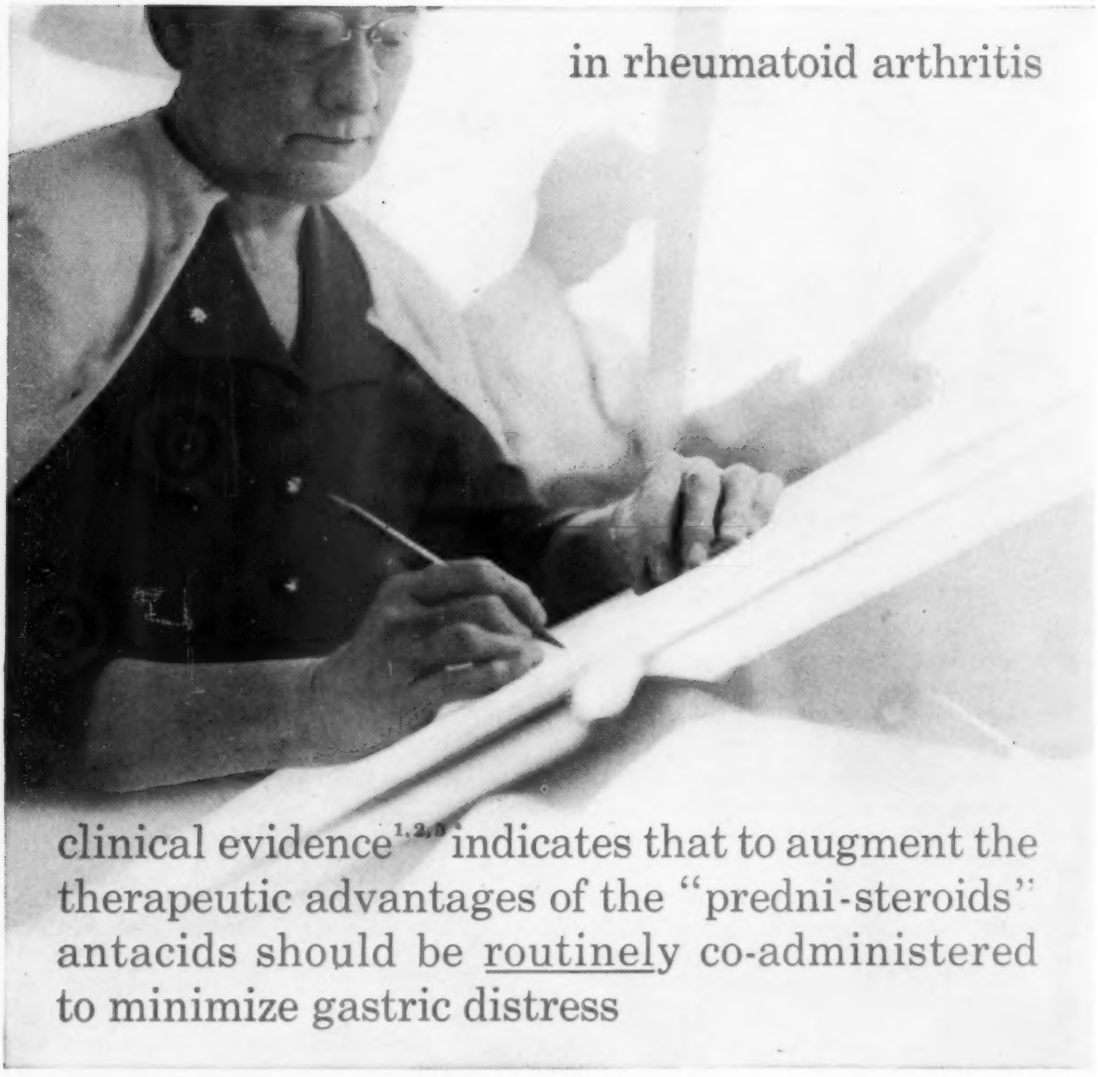

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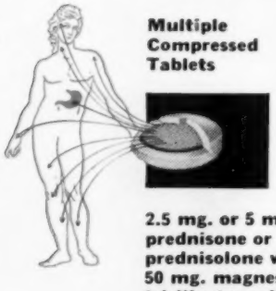

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SQUIBB *Squibb Quality—the Priceless Ingredient*

*Erythromycin in the treatment of osteomyelitis**

8/3/55

CASE SUMMARY

On 6/2/55, patient, male, age 28, fell on an old fracture and refractured the middle third of the right femur, superimposed on an old osteomyelitis.

On 7/7/55, the wound was saucerized and a hemolytic S. aureus (coag. +) was isolated from the osteomyelitis. Disc sensitivities were: penicillin, 10 units; erythromycin, 10 mcg.; tetracycline, 10 mcg.

On 7/15, the patient was placed on erythromycin therapy 400 mgm. q. 6. h. Patient afebrile after erythromycin started. X-rays showed evidence of healing with callus formation. No septicemia and clinical evidence indicates control of the infection.

On 8/3, the cast was removed and leg recast. Wound was in good condition with minimal drainage.

Diagnosis: fracture middle third of right femur, complicated by osteomyelitis.

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The Importance of Rescinnamine in

Rauwiloid®

The Original Alseroxylon Fraction of India-Grown Rauwolfia Serpentina, Benth.

The isolation of rescinnamine,[1] another potent alkaloid in Rauwolfia serpentina, has substantiated two important points:

A—It discredits the erroneous opinion that reserpine is *the* sole active principle of Rauwolfia;[2]

B—It helps to define the advantages of Rauwiloid, the alseroxylon fraction of Rauwolfia serpentina, which presents desirable alkaloids[3] of the Rauwolfia plant (among them reserpine and rescinnamine) but is freed from undesirable alkaloids and the dross of the crude root.

Pharmacologic and clinical evaluation has shown rescinnamine to be similar to reserpine in antihypertensive activity, but to be considerably less sedative and much less apt to lead to lethargy and mental depression.[4, 5]

The interaction of reserpine, rescinnamine, and other contained alkaloids may well account for the balanced and desirable clinical behavior of Rauwiloid.

The dosage of Rauwiloid is simple and definite: Merely two 2 mg. tablets at bedtime. For maintenance, one tablet usually suffices.

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2. Cronheim, G.; Brown, W.; Cawthorne, J.; Toekes, M.I., and Ungari, J.: Pharmacological Studies with Rescinnamine, a New Alkaloid Isolated from Rauwolfia Serpentina, Proc. Soc. Exper. Biol. & Med. 86:120 (May) 1954.
3. Gourzis, J.T.; Sonnenschein, P.R., and Barden, R.: Alterations in Cardiovascular Responses of the Dog Following Rauwiloid, an Alkaloidal Extract of Rauwolfia Serpentina, Proc. Soc. Exper. Biol. & Med. 85:463 (Mar.) 1954.
4. Hershberger, R.; Hughes, W., and Dennis, E.: Clinical Results in the Treatment of Hypertension with Rescinnamine, Clin. Res. Proc. J:71 (Feb.) 1955.
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instead of these risks...

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Aluminum hydroxide	2.5 gr.
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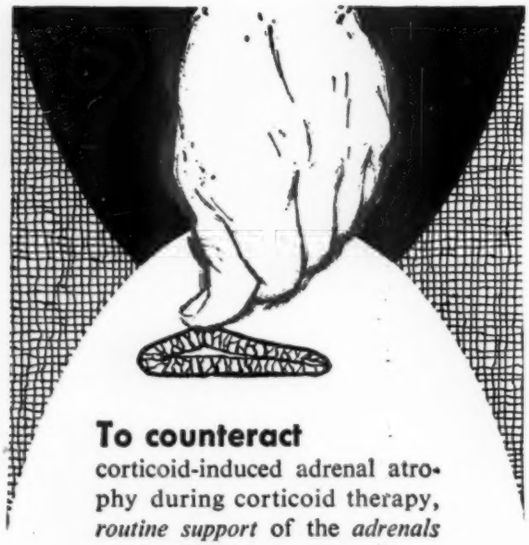

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- When using *prednisone* or *prednisolone:* for every 100 mg. given, inject approximately 100 to 120 units of HP* ACTHAR *Gel.*
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- When using *cortisone:* for every 400 mg. given, inject approximately 100 units of HP*ACTHAR *Gel.*

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Riboflavin (B_2)	10 mg.
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Pyridoxine HCl (B_6)	5 mg.
Sodium Pantothenate	10 mg.
Ascorbic Acid (C)	300 mg.
Vitamin B_{12}	15 mcgm.
Folic Acid	3 mg.

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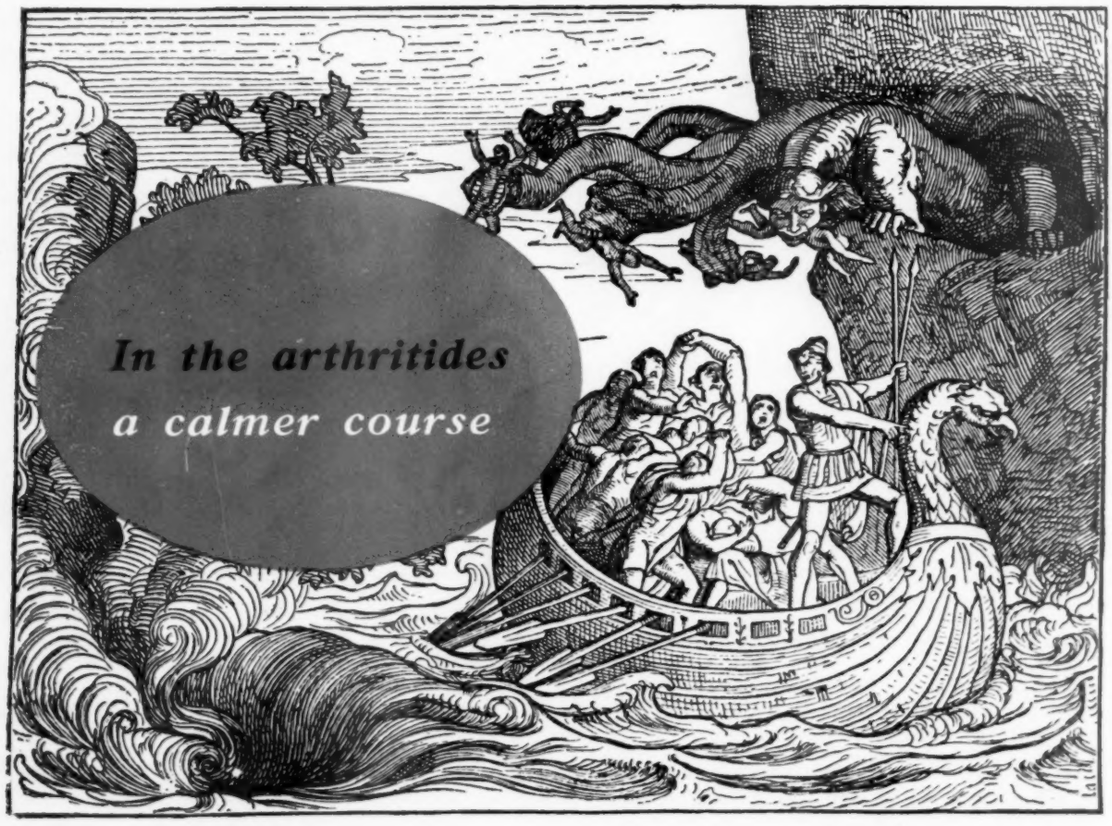

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Because of the complementary action of cortisone and the salicylates, Salcort produces a greater therapeutic response with lower dosage. Side effects are not encountered, and no withdrawal problems have been reported.

One study concludes: "Salicylate potentiates the greatly reduced amount of cortisone present so that its full effect is brought out without evoking undesirable side reactions."[1]

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Calcium ascorbate. . . . (equivalent to 50 mg. ascorbic acid)	60.0 mg.
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[1] *Busse, E.A.: Treatment of Rheumatoid Arthritis by a Combination of Cortisone and Salicylates. Clinical Med.* 11:*1105*

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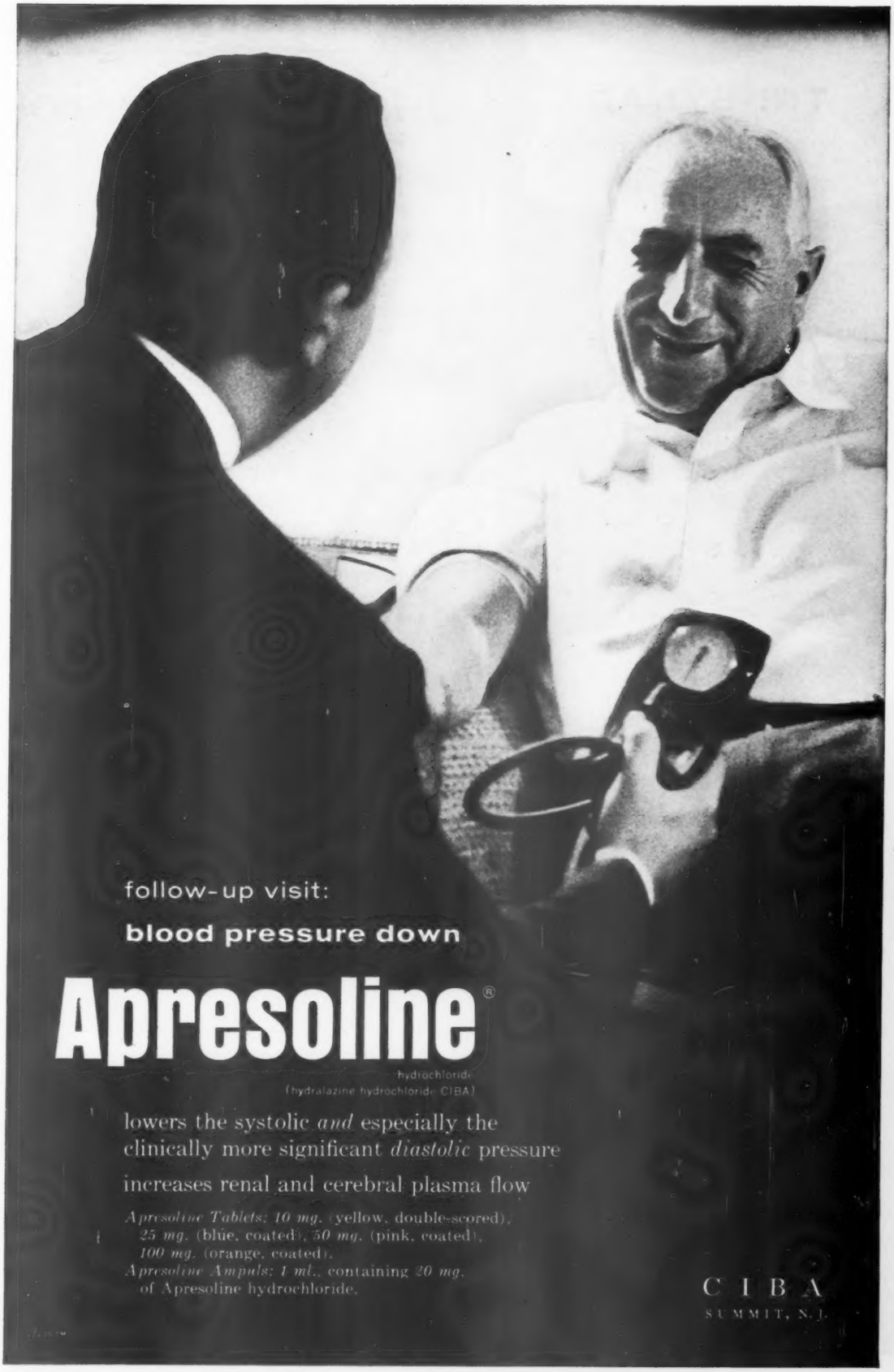
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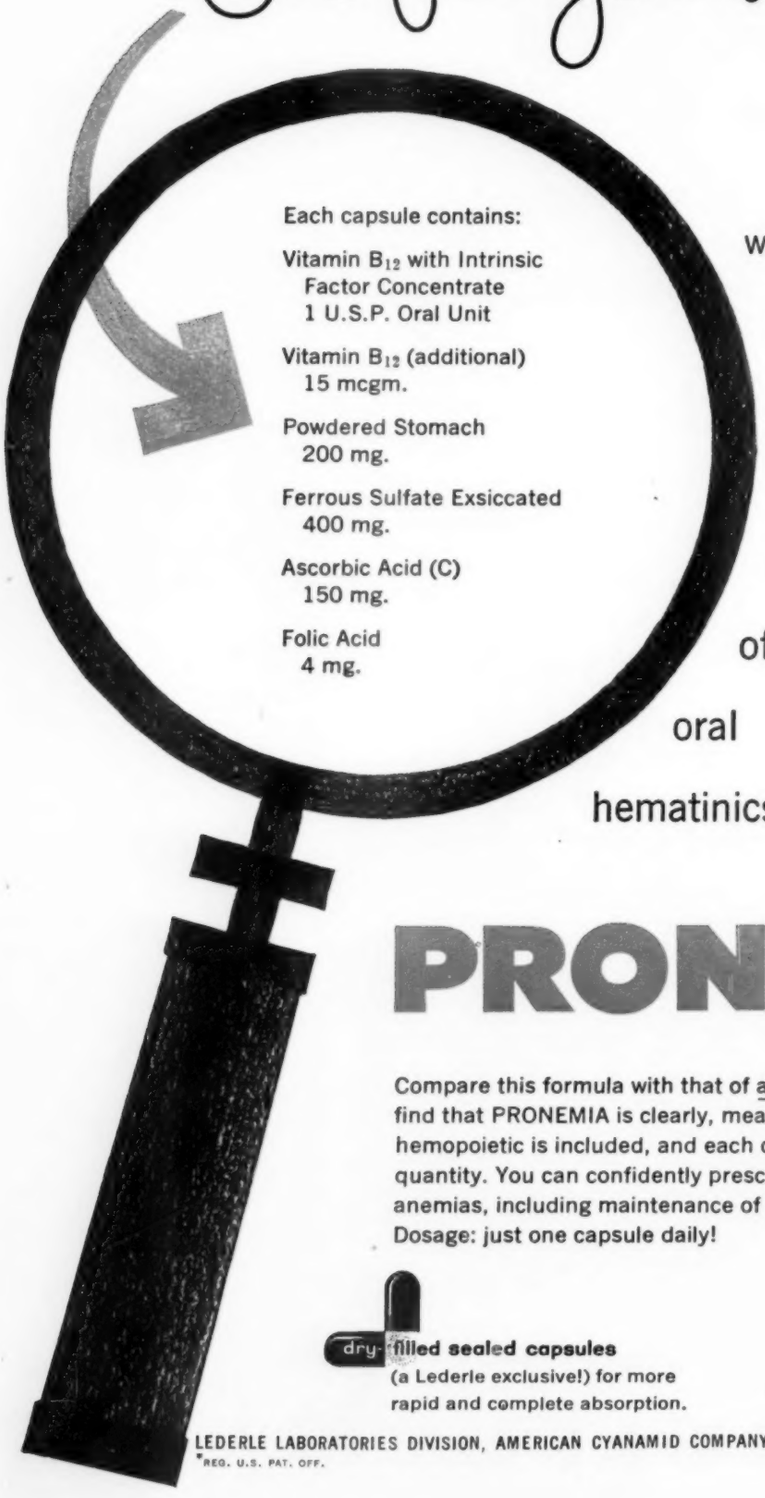

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relieves pain

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NOW 50% more Ferrous Sulfate
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ALSO ADDED: ½ U.S.P. Unit of Vitamin B_{12} with intrinsic factor concentrate

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Folic acid U.S.P.	1 mg.
Thiamine mononitrate (B_1)	10 mg.

Supplied

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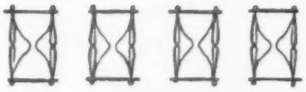
for 4 full hours 

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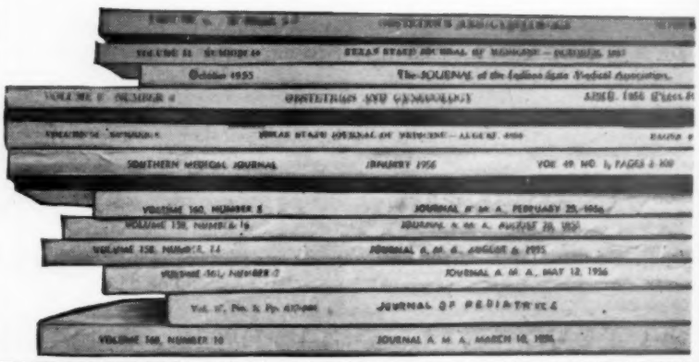

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1. Eisfelder, H.W.: Am. Pract. & Dig. Treat., 5:778 (Oct.) 1954).
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Reference: 1. Pocock, D. G.: Personal communication.

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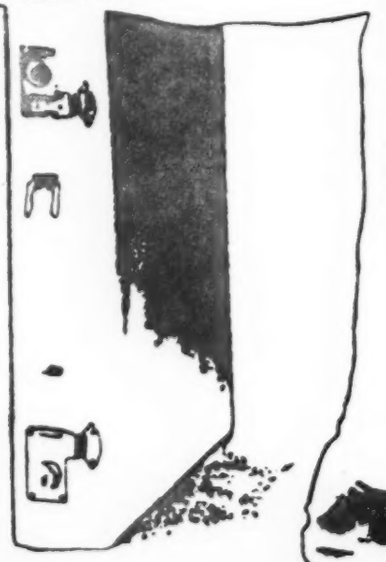

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